Thrombin and Fibrinolysis*

Michael Nesheim, PhD

When the activities of the coagulation and fibrinolytic cascades are properly regulated, so that fibrin (FN) deposition and removal are properly balanced, the vascular system is protected from catastrophic blood loss at the site of an injury, while its fluidity is ensured elsewhere. When these activities are not properly regulated, however, the organism is subjected to either excessive bleeding or thrombosis. Thrombomodulin on the endothelial cell is very important in this regulation because it converts thrombin to an anticoagulant enzyme by directing it toward the activation of protein C. It also converts thrombin to an anti-fibrinolytic enzyme by directing it toward the activation of thrombin-activatable fibrinolysis inhibitor (TAFI). By doing so, it creates a direct molecular connection between the coagulation and fibrinolytic cascades, such that activation of the former suppresses the activity of the latter. Recent studies indicate that the TAFI pathway functions in vivo and is likely relevant in maintaining the proper balance between FN deposition and removal. Whether it will be a target for pharmaceutical manipulation of this balance remains to be determined.

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Abbreviations: FDP = fibrin degradation product; FN = fibrin; FPA = fibrinopeptide A; FPB = fibrinopeptide B; GPg = glu-plasminogen; GPn = glu-plasmin; LPg = lys-plasminogen; LPn = lys-plasmin; PAI-1 = plasminogen activator inhibitor type 1; PTCI = potato tuber carboxypeptidase inhibitor; TAFI = thrombin-activatable fibrinolysis inhibitor; TAFIa = carboxypeptidase B-like enzyme; tPA = tissue-type plasminogen activator

The ongoing deposition and removal of fibrin (FN) both protects the organism from untoward blood loss at the site of injury and maintains the fluidity of the blood elsewhere.1–4 FN deposition occurs on activation of the coagulation cascade. This results in the ultimate conversion of prothrombin to thrombin, which then catalyzes conversion of soluble fibrinogen to the gel-like substance, FN, the major proteinaceous component of the clot (Fig 1). FN removal occurs on activation of the fibrinolytic cascade. Plasminogen then converts to the enzyme plasmin, which catalyzes digestion of the FN clot to soluble FN degradation products.

The activities of the cascades are normally latent. Their potentials, however, have been shown in an animal model (chimpanzee) to be remarkable.5 The coagulation cascade, for example, when acutely stimulated, can convert the entire plasma content of fibrinogen to FN and force the level of circulating platelets to zero in less than a minute. This potentially lethal phenomenon, however, is matched by an equally potent fibrinolytic response, such that within a minute or two, the plasma level of endogenous tissue plasminogen activator increases several hundred fold, the deposited FN is completely solubilized, and the circulating platelet level returns to normal. All of this occurs without long-term negative effects on the animal. Thus, both the coagulation and fibrinolytic cascades have the potential to generate very profound activities in a very acute manner. When the processes of FN deposition and removal are properly regulated, they perform their physiologic functions remarkably well. When they are not properly balanced, however, the pathophysiologic consequences are reflected in tendencies to bleed or thrombose. Prevalent thrombotic events include heart attacks and strokes.

The regulation of the function of the cascades is expressed through the endothelium, the blood platelets, and the coagulation and fibrinolytic plasma proteins. One key player, indicated in Figure 1, is thrombomodulin,6 an integral membrane protein found on the endothelial cell. It binds to thrombin and changes its substrate specificity, such that it no longer recognizes fibrinogen as a substrate. Instead, it catalyzes conversion of the zymogen, protein C, to the anticoagulant enzyme, activated-protein C. This enzyme inactivates factors Va and VIIIa of the coagulation

![Diagram of coagulation and fibrinolytic cascades](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21999/ on 06/27/2017)
cascade and thereby down-regulates thrombin formation. This subject is discussed in detail in the article by Dr. Esmon in this supplement. In addition, thrombin, particularly when it is bound to thrombomodulin, catalyzes the activation of the zymogen thrombin-activatable fibrinolysis inhibitor (TAFI) to the antifibrinolytic, carboxypeptidase B-like enzyme (TAFIa), which down-regulates the cofactor activity of FN in plasminogen activation and thereby suppresses fibrinolysis. The pathway defined by thrombin, thrombomodulin, and TAFI creates a direct molecular connection between the coagulation and fibrinolytic cascades, such that activation of the former suppresses the activity of the latter. Thus, this pathway may play a key role in the regulation of the balance between FN deposition and removal.

**THE FIBRINOLYTIC CASCADE**

The formation of FN from fibrinogen is depicted in Figure 2. The fibrinogen molecule comprises two globular D domains and a central, smaller, globular E domain. Thrombin catalyzes removal of fibrinopeptide A (FPA) and fibrinopeptide B (FPB) from the molecule. These events expose polymerization sites within the E domain such that E domains associate specifically, tightly, and noncovalently with sites on the D domains of neighboring molecules to initiate the polymerization. Protofibrils consisting of two linear polymers form in a half-staggered arrangement. Simultaneously, factor XIII is activated to factor XIIIa, and it catalyzes covalent isopeptide bonds between γ chains in the D domains of adjacent FN monomers within the growing polymer. With further liberation of FPA and FPB, double-stranded protofibrils laterally associate to make FN bundles, which extend in length and occasionally form branches. As a result, a FN mesh develops that resembles a “three-dimensional spider web.” The solution in which this occurs becomes gel-like and forms, along with entrained cells, the familiar blood clot. In addition, a carboxyl terminal arginine is removed from FPB by a carboxypeptidase B-like enzyme. The FN clot is subsequently digested when the fibrinolytic cascade is triggered. These events are depicted in Figure 3. When FN forms, it serves as a cofactor for the conversion of glu-plasminogen (GPg) to glu-plasmin (GPn). This process begins digestion of FN by catalyzing cleavage after specific arginine and lysine residues in the α, β, and γ chains of FN. The FN, while still clotted, is modified to a form designated FN’ in Figure 3. This modified form has in its structure carboxyl terminal lysine residues, which promote the binding of tissue-type plasminogen activator (tPA) and GPg to FN. This, in effect, up-regulates the fibrinolytic cascade, the cascade responds to FN generated by the action of thrombin on fibrinogen, tPA is released from the endothelium and, with FN as a cofactor, catalyzes conversion of GPg to GPn, which begins to digest FN to a modified form (FN’) with increased cofactor activity. FN’ also serves as a cofactor for the plasmin-catalyzed conversion of GPg to LPg, which is a better substrate than GPg for tPA. This reaction and the conversion of FN to FN’ represent positive feedback in the fibrinolytic cascade. When TAFIa is formed, it further modifies FN’ to FN” and thereby eliminates positive feedback and attenuates fibrinolysis. The cascade is also down-regulated by the serine protease inhibitors, PAI-1, and antiplasmin (AP), which irreversibly inactivate tPA and plasmin, respectively. TM = thrombomodulin.
cofactor activity of FN’ to a level threefold higher than that of intact FN. FN’ also serves as a cofactor for the proteolytic conversions of GPg and GPn to their lysine counterparts, lys-plasminogen (LPG) and lys-plasmin (LPn). These reactions are catalyzed by GPn and LPn. LPG is approximately 20-fold better than GPg as a substrate for TPA-catalyzed formation of plasmin (LPn). Thus, modification of FN to FN’ represents positive feedback in the fibrinolytic cascade, somewhat akin to that represented by thrombin-catalyzed activation of factors V and VIII in the coagulation cascade by thrombin. In response to thrombin, and especially thrombin bound to thrombomodulin, TAFI is activated to TAFIa. TAFIa then catalyzes removal of the carboxyl terminal lysine (arginine) residues present in FN’, thereby producing a form of modified FN designated FN”.

This down-regulates the cofactor activity of FN with respect to both the activation of GPg and the conversion of GPg to LPG. Thus, TAFIa, while not eliminating plasminogen activation, eliminates the positive feedback steps and thereby substantially attenuates plasminogen activation and fibrinolysis. The fibrinolytic cascade is also down-regulated by two fast-acting serine protease inhibitors. One of these is plasminogen activator inhibitor type 1 (PAI-1), which targets tPA. The other is antiplasmin, which targets GPn and LPn. Interestingly, FN reduces the rate of inhibition of plasmin by antiplasmin by a factor of approximately 100. The fibrinolytic process terminates in the digestion of FN’ and FN” to a family of soluble FN degradation products (FDPs), the smallest of which (DDE) is shown in Figure 3.

The coagulation and fibrinolytic cascades have similar general features in common. They both respond to an activator liberated from the tissues (tissue factor in coagulation) or the endothelium (tPA in fibrinolysis), they both proceed by zymogen to protease conversions, they both have positive feedback steps and therefore generate explosive activity following an initiation phase, and they are down-regulated by both protease inhibitors and reactions mediated by the thrombin-thrombomodulin complex.

**TAFI Protein and TAFI Gene**

TAFI is a single-chain plasma protein of 401 amino acids. Its peptide molecular mass is 45,999 d. In gel electrophoresis in dodecyl sulfate, it migrates with an apparent molecular weight of 60,000 d, a feature that can be attributed to four glycosaminoglycan chains in the amino-terminal region of the molecule. It circulates in plasma at a concentration of approximately 100 nmol (6 μg/mL), a value similar to that of protein C. The plasma level of TAFI appears to be strongly determined by polymorphisms in the promoter and 3’ untranslated region of the gene. It is cleaved after arginine 92 by thrombin, thrombin-thrombomodulin, and plasmin to yield a 92-amino acid activation fragment from the amino terminus and the carboxypeptidase B-like enzyme, TAFIa, from the carboxyl terminus. The enzyme comprises 309 amino acids and migrates in gel electrophoresis in dodecyl sulfate with an apparent molecular weight of 35,000 d. The enzyme is highly homologous to the pancreatic carboxypeptidase-B.

Figure 4. Mechanism of TAFI activation. TAFI can be generated from the binary thrombin-TAFI complex (T-TAFI) or the ternary thrombin-thrombomodulin-TAFI complex (T-TM-TAFI). Kd and Km values are the dissociation constants for the corresponding interactions, and k1 and k2 are turnover numbers for TAFIa generation. The catalytic efficiency for the thrombin-dependent reaction is 1,250-fold greater than that for the thrombin-thrombomodulin-independent reaction.
thrombomodulin is required for TAFI activation. In addition, regions of thrombin that are sufficient for the two reactions differ. Furthermore, the thrombomodulin dependence of TAFI activation is not determined by the amino acids surrounding the cleavage site. One particularly notable difference involves methionine 388 of thrombomodulin. This residue is highly susceptible to oxidation, a reaction that can be accomplished by inflammatory cells. Oxidation of this residue reduces the effectiveness of thrombomodulin in protein C activation by approximately 90% but does not affect TAFI activation. This observation prompts the speculation that the balance between FN deposition and FN removal might be tipped strongly toward thrombosis in an inflammatory milieu due to the loss of the anticoagulant but not antifibrinolytic properties of thrombomodulin.

The enzyme, TAFIa, is intrinsically unstable. The kinetics of decay are highly dependent on temperature. At body temperature the enzyme has a half-life of approximately 8 min. The antifibrinolytic potential is directly related to the half-life. Since no naturally occurring inhibitors of TAFIa have been found in the plasma, the intrinsic instability appears to reflect the physiologic mechanism for down-regulation. The region of the molecule associated with instability has been shown to encompass amino acids 302–330. A common polymorphism in the human population has been found at position 325. One form exhibits threonine at this position and the other isoleucine. The latter form is twice as stable and 60% more potent as an antifibrinolytic. Whether this fact has pathophysiologic consequences, however, has yet to be determined, although a recent report indicates that it is not associated with a tendency for myocardial infarction.

Fibrinolysis and the Intrinsic Pathway of Coagulation

A comprehensive analysis has been made of the temporal course of events associated with the clotting of whole blood when the process is initiated through the tissue factor-dependent (extrinsic) pathway. The process begins with an initiation phase, which lasts approximately 4.5 min. In this phase, thrombin at low levels catalyzes in sequence platelet activation, factor V activation, factor XIII activation, and FPA release. Over this interval, the thrombin level reaches approximately 25 nmol (2.5 U/mL). At the end of this phase, clotting occurs (4.7 min). In blood from normal individuals, the initiation phase is immediately followed by a propagation phase in which the intrinsic pathway is triggered and a relatively enormous burst of thrombin occurs within the clot, peaking at a concentration of approximately 550 nmol. This is then consumed fairly rapidly over the next few minutes by antithrombin and other protease inhibitors. In blood from people with deficiencies in the intrinsic pathway (e.g., hemophilia A or B), the initiation phase is similar to that of blood from normal individuals, although clotting is delayed modestly. Strikingly, however, the large burst of thrombin following clotting is extremely diminished or absent. The dire bleeding tendencies in hemophilia A and B do not seem to be rationalized very well by modest deficiencies in the initiation phase. Rather, they seem to correlate more obviously with the failure to produce the propagation phase. A logical conundrum still exists, however, because clotting is associated with the initiation phase, which is nearly normal in hemophilic blood. Studies have shown, however, that plasmas deficient in components of the intrinsic pathway (factors VIII, IX, and XI, but not XII), when clotted via the extrinsic pathway, in the presence of tPA, exhibit subsequent fibrinolysis three times earlier than normal plasmas. The phenomenon has been designated "premature lysis." The mechanism behind this phenomenon has been worked out. In normal plasma, the burst of thrombin following clotting is sufficient, even in the absence of thrombomodulin, to activate sufficient TAFI to suppress subsequent plasminogen activation and fibrinolysis. This does not happen in the hemophilic plasma, however, because of the lack of the thrombin burst in the propagation phase. Consequently, the clots formed in the initiation phase dissolve more quickly. The concepts are presented in Figure 5. This premature lysis to date has only been demonstrated in vitro. Its existence, however, has led to the hypothesis that bleeding in patients with defects in the intrinsic pathway may be as much due to the failure to suppress fibrinolysis as it is to a failure to form a clot in the first place.

![Figure 5: Suppression of fibrinolysis through the intrinsic pathway](image)

Thrombin: Physiology and Pathophysiology
Some Potential Clinical Implications of the TAFI System

Insufficient time has passed since the discovery of the TAFI system to have acquired enough information to determine definitively whether it will be relevant with respect to pathophysiology, diagnosis, or treatment. Epidemiologic studies and work in animals, however, suggest that it might. For example, elevated levels of plasma TAFI have been associated with a mildly elevated risk for venous thrombosis, and it is significantly elevated (50%) in patients with type 2 diabetes. In a large cohort studied in France, an increase in the TAFI antigen level was found to be a risk factor for angina pectoris; interestingly, the same result was not seen in a study in Northern Ireland. TAFI levels measured both by antigen detection and functional assays were found to be significantly lower than normal in disseminated intravascular coagulation. Similar measurements in acute promyelocytic leukemia showed that antigen levels were normal, but levels measured on the basis of function were reduced approximately 60%. The suggestion made in the report of this study was that the acquired TAFI deficiency in acute promyelocytic leukemia may contribute to the severity of the hemorrhagic diathesis in this disease because of an impairment in the capacity of the coagulation system to protect the clot from the fibrinolytic system. Levels of TAFI were found to be very low in patients with liver cirrhosis and even undetectable in patients with advanced hepatocellular disease. Recombinant factor VIIa, used for therapy of hemophiliacs with inhibitors to factor VIII, was shown to prolong clot lysis in vitro. The effect was shown to be TAFI dependent. The authors concluded that recombinant factor VIIa both accelerates clot formation and inhibits fibrinolysis in factor VIII-deficient plasma. They also reported a large individual variability in antifibrinolytic potential of recombinant factor VIIa in the population studied. These studies and others like them all suggest that TAFI may be linked to certain pathologic conditions, but to date no pathologic condition has been identified that can be linked directly to a defect in the TAFI system.

Studies in animal models show that TAFIa functions as an antifibrinolytic agent in vivo. Early work by Redlitz et al. showed that a carboxypeptidase B-like activity is induced when thrombosis is initiated in a canine thrombolytic model. The activation of TAFI in another dog model of coronary artery thrombosis was shown to be inhibited by the thrombin inhibitor melagatran. Klement et al. investigated the impact of a TAFIa inhibitor on tPA-induced clot lysis in a rabbit model of arterial thrombosis. The inhibitor was a peptide isolated from the potato called potato tuber carboxypeptidase inhibitor (PTCI). They found that when the inhibitor was administered along with tPA, numerous parameters associated with thrombolysis were enhanced. The time to reperfusion was reduced by an approximate factor of three. Vessel patency was similarly improved. In their experiments, they measured both clot accretion and lysis. The TAFIa inhibitor did not influence accretion but it substantially promoted clot lysis. Similar studies were carried out by Nagashima et al. in a rabbit model of venous thrombolysis, where the impact of PTCI on tPA-mediated clot lysis was determined. Indwelling clots were removed and weighted 90 min after initiation of the thrombolytic treatment, which consisted of a bolus of tPA, with or without PTCI, followed by continuous infusion of tPA, with or without PTCI. They found that the weight of the thrombus was not affected by PTCI when it was administered along with a saline solution control. When it was administered along with tPA, however, the clot weight was reduced to approximately one half of the control weight. tPA alone at the same dose reduced clot weight only 26%. This combined effect of tPA and PTCI could be obtained with three times the dose of tPA in the absence of PTCI. Thus, the TAFIa inhibitor effectively tripled the effect of a given dose of tPA. In addition, when PTCI was administered along with the higher dose of tPA, the clots were completely digested. A similar apparent tripling of the effectiveness of tPA was reported by Refino et al in another animal model of thrombolysis. These studies suggest that TAFIa inhibitors could be fruitfully employed as adjuvants in thrombolytic therapy.

Whether drugs will inhibit TAFIa or suppress TAFI activation in order to promote endogenous fibrinolysis and thereby offer long-term protection from undesirable thrombotic events is not yet known. To date, no work in animals along these lines has been reported. Most work in vitro, however, indicates that TAFIa modulation affects the balance between FN deposition and removal, and thereby provides solid rationale for pursuing the appropriate investigations. The TAFI knockout mouse has been obtained. The mice are viable, and the absence of TAFI in this species does not create a phenotype, which suggests that TAFIa inhibition would not be unacceptably dangerous.

In the case of hemophilia, a reasonable argument can be made that an agent that would enhance TAFI activation might help alleviate bleeding. An obvious choice for such an agent would be a soluble recombinant thrombomodulin engineered to promote TAFI activation but not protein C activation. In vitro, soluble thrombomodulin has been shown to correct the premature lysis of clots in hemophilia plasma, because in the presence of thrombomodulin the thrombin generated in the initiation phase is adequate to activate TAFI, even in the absence of the thrombin burst, which occurs in normal plasma during the propagation phase. Since the elements of thrombomodulin structure needed to activate TAFI and protein C differ somewhat, the acquisition of a form of thrombomodulin selective for TAFI activation is feasible. In fact, two potential variants are currently known. One with oxidized methionine 388 is effective with TAFIa but not with protein C. In addition, a construct with tyrosine 348 replaced with alanine functions normally in TAFI activation but not protein C activation.

Whether measurements of TAFI, TAFIa, or other proteolytic fragments will prove useful for diagnosis, prognosis, or risk assessment is not yet known. This is also true for analyzing numerous polymorphisms in both the translated and untranslated portions of the TAFI gene. Several enzyme-linked immunosorbent assays are available commercially, however.
Thrombin: Physiology and Pathophysiology

Summary

The coagulation and fibrinolytic cascades are generally thought of as separate and independent entities. Recent observations, however, suggest that they are directly linked to one another. This occurs not only through the TAFI pathway, but also through reactions whereby plasmin, for example, can both activate and/or inactivate coagulation factors V and IX. Consequently, methods for more efficacious control of bleeding or thrombosis will likely be obtained from insights gained through studies aimed at acquiring a more global appreciation of the mechanisms involved in regulating not just coagulation or fibrinolysis, but rather in regulating the balance between these two important physiological processes.

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It is relatively short half-life would suggest that elevated TAFI levels would reflect events that are currently happening, rather than those that happened in the past.

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