Glycosylation of Thrombin Activatable Fibrinolysis Inhibitor
Why Is it So Important?
Federico Biscetti

The coagulation and fibrinolytic systems safeguard the patency of the vasculature and the surrounding tissue. Regulation of these systems is accomplished by various mechanisms involving cellular responses, flow, and protein–protein interactions. Thrombin activatable fibrinolysis inhibitor (TAFI) is a fibrinolysis inhibitor, and its activation is sensitive to the dynamics of the coagulation system; it is one of the main intermediaries between coagulation and fibrinolysis. TAFI is a thrombin, thrombin/thrombomodulin and/or plasmin-activated enzyme that has greatly improved our understanding of the cross-regulation between coagulation and fibrinolysis, and its pathway modulates both the coagulation and the fibrinolytic system.1,2

TAFI was identified at the same time by different groups, and this resulted in initial different names for the same protein3–6: procarboxypeptidase U (pro-CPU), plasma procarboxypeptidase B (propCPB), and procarboxypeptidase R (pro-CPR). At the moment, TAFI is the most commonly used denomination.

TAFI is synthesized in the liver and secreted as a propeptide consisting of 401 amino acids.2 It has a molecular mass of 56 kDa. TAFI is activated by plasmin, by thrombin generated via the extrinsic or intrinsic coagulation pathways, or in presence of thrombomodulin.7–10 Activated TAFI (TAFIa) inhibits fibrinolysis by modulating the fibrin cofactor function for plasmin generation. Proteolysis of C-terminal lysine residues of fibrin by TAFIa abrogates the fibrin cofactor function for tissue plasminogen activator–mediated plasminogen activation resulting in a plasmin formation suppression.11

In recent years, there has been increasing appreciation of the fact that recombinant TAFI protein may have important therapeutic implications. In fact, antifibrinolytic therapy using TAFIa may help prevent bleeding manifestations in several pathological conditions, such as hemophilia. The enzymatic activity of TAFIa, however, is unstable and sensitive to temperature, and TAFIa inactivation is caused by conformational stochastic changes.12 Construction of TAFI chimeras (such as TAFI-containing residues of pancreatic carboxypeptidase B, a stable protease) increase the half-life of TAFIa enzymatic activity, but its antifibrinolytic activity is compromised.13 Glycosylation may augment the activity and the antifibrinolytic potential of TAFI.

In the article published by Buelens et al14 in this issue of Circulation Research, the authors report the construction, expression, and characterization of several mutants of TAFI missing 1 or more N-linked glycosylation sites. This type of posttranslational modification may also influence many of the biochemical properties of the proteins, such as stability, dynamics, and ligand binding.15,16 N-linked glycosylation is needed to stabilize folded domains and to provide solubility by enhancing polar surface groups, and it is an important posttranslational modification for the folding of many of the coagulation proteins. In fact, the overall extent of glycosylation is a primary determinant of antithrombin clearance17; in addition, the heterogeneity of FV1–FV2, which are the 2 circulating isoforms of coagulation factor V, is caused by differential glycosylation of Asn2181, related to the presence of Ser rather than Thr in the third position in the consensus sequence of glycosylation.18 Furthermore, the different biochemical properties of glycosylated and nonglycosylated plasminogen activator inhibitor-1 depends specifically on glycosylation of N209 or N265 sites, and the plasminogen activator inhibitor-1–binding protein, vitronectin, reverses the changes associated with the lack of glycosylation at one of these sites.19 Again, glycosylation alone does not lead to higher secretion rates; it must occur in the context of the normal structure of the FVIII coagulation protein.20 Moreover, N-glycosylation is crucial during the normal processing of human coagulation factor VII.21 Finally, N-linked glycosylation is important in determining molecular weight, thrombin cleavage, and functional activity of human protein S.22 Using an elegant approach, Buelens et al14 have determined the biochemical importance of the glycosylation of TAFI. From their data it can be concluded that it is mainly the glycosylation at Asn86 that contributes to the biochemical properties of TAFI. Furthermore, the authors provide evidence that the activation peptide stays in close proximity to the TAFIa moiety after activation (Figure).

Cleavage of TAFI by thrombin, thrombin/thrombomodulin, and plasmin leads to the formation of an activation peptide (20 kDa) and of a catalytic domain TAFIa (36 kDa). N-glycosylation is a major posttranslational modification, and it takes place before the protein is folded.23 The biochemical importance of glycosylation of TAFI has not yet been clarified. It seems that the inherent instability of TAFIa is not caused by posttranslational modifications but that glycosylation is needed to stabilize the protein and that, after activation, TAFIa becomes less soluble on cleavage of the glyco-

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Figure. A schematic representation of the activation and function of TAFI. The initial step, before its cleavage to TAFIa by thrombin, thrombin/thrombomodulin, or plasmin, is the glycosylation at Asn86.

 Glycosylated activation peptide. A number of biochemical properties (eg, TAFIa activity, enzyme kinetics, and antifibrinolytic potential) of TAFI are solely attributed to the TAFIa. Buelens et al demonstrate that these properties are altered when the glycosylation of the activation peptide is altered and that glycans may assume a crucial role in TAFI functions. These findings provide new information for the understanding of the biological and clinical role of TAFI. It is also possible to hypothesize that these issues may be important for recombinant TAFI protein construction, with potentially important therapeutic implications for the management of patients affected by pathological bleeding conditions.

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