by Dr. Jovan P. Antovic

Until recently, coagulation and fibrinolysis were considered as separate entities linked by fibrin, the final product of the coagulation system which serves as a substrate for the fibrinolytic system. Accounts of thrombin activatable fibrinolysis inhibitor (TAFI) have shed more light on this field and it has been proposed that this enzyme may form another link between the two systems.

Thrombin activatable fibrinolysis inhibitor (TAFI): a link between coagulation and fibrinolysis

In 1989, a labile carboxypeptidase activity that interferes with carboxypeptidase N activity was described. This entity was not present in blood, but appeared after clotting. Bajzar and collaborators purified the same enzyme and demonstrated that it could be activated by thrombin, and is also capable of inhibiting fibrinolysis. Consequently this enzyme was named thrombin activatable fibrinolysis inhibitor (TAFI). This enzyme has since been given a number of different names, including carboxypeptidase U (unstable), carboxypeptidase R (the enzyme cleaves arginine residues) and plasma carboxypeptidase B (the enzyme shares similarities with pancreatic carboxypeptidase B). The most exact numerical nomenclature for this enzyme, EC 3.4.17.3, is not convenient for everyday use, thus a different nomenclature based on the widely accepted terminology for coagulation proenzymes and their active forms has been suggested. This nomenclature also provides a link between the enzyme’s name and function as suggested by Bajzar and collaborators. According to this new nomenclature, the precursor of TAFI (zymogen) is designated pro-TAFI. Since this inhibitor of fibrinolysis is functional only in its active form, there is no point in using the term TAFI. Instead it has been suggested that the term TAFI should be reserved for the enzyme’s active form. An inactive form of TAFI also exists and this is termed TAFII [Table 1].

Synthesis, characterisation and purification of TAFI

Pro-TAFI is a glycoprotein synthesised in the liver as a prepropeptide consisting of 423 amino acids with a molecular weight of 55 kD. The plasma concentration of pro-TAFI ranges from 4 µg/mL to 15 µg/mL and it circulates bound to plasminogen. Pro-TAFI has recently also been identified in platelets. TAFI antigen has not been found in cerebrospinal fluid and there is currently no data to suggest that TAFI is present in other human body fluids.

Purification of pro-TAFI is based on its high affinity for plasminogen. Plasma is applied to a lysine-Sepharose column, which removes plasminogen and proteins bound to plasminogen. This process is followed by affinity chromatography on plasminogen-Sepharose and subsequent elution of pro-TAFI by epsilon amino caproic acid (eACA). Ion exchange chromatography is also frequently used.

Genomic organisation and TAFI polymorphisms

The pro-TAFI gene is 48 kb in size, consisting of 11 exons, and maps to chromosome 13q14.11. A pro-TAFI deficiency has not yet been described in humans, which may indicate that it is either of no significance, or would be incompatible with life. Knock-out mice for the pro-TAFI gene have been developed. No embryonic lethality was observed, and deficient mice developed normally, reached adulthood and were fertile with normal pregnancies. Pro-TAFI deficiency did not lead to an increased bleeding tendency or influence the thrombosis model.

Several forms of the pro-TAFI gene have been described. An alanine-threonine substitution can occur at position 147, however, this does not lead to significant changes in TAFI function. Eleven further examples of polymorphisms have been found (5 in the promoter: C-2599G, -23452G/-1G, A-1690G, G-1102T and G-438A, 2 in the 3’ region: C+1542G and T+1583A and 4 in the 5’ untranslated region -152A/G, -530C/T, -1053T/C and -1925T/C) and individually these contribute to a large fraction of the TAFI antigen level. The role of the different forms of TAFI and their influence on TAFI levels, particularly with respect to clinical conditions, have not yet been established. However, it has been shown that TAFI levels are altered significantly when the -438A/G and 1040C/T polymorphisms are present.

Activation of pro-TAFI

Pro-TAFI is activated by trypsin, plasmin and thrombin after cleavage at Arg92 to yield a 15 kD activation peptide and a 35 kD activated enzyme, TAFI. Thrombin is a poor activator of pro-TAFI, with a kcat of 0.0021 s⁻¹. Plasmin activates pro-TAFI eight times more efficiently. Thrombomodulin enhances the thrombin-dependent activation of pro-TAFI 1250-fold, primarily through the increase in kcat, indicating that the thrombin/thrombomodulin complex is probably the main physiological activator of pro-TAFI. However, it has also been shown that TAFI activity displays a biphasic pattern, peaking first during coagulation and then during the fibrinolytic phase. This implies that both thrombin and plasmin may generate TAFI.

Inactivation of TAFI

TAFI is highly unstable, with a half-life of only 10 minutes at 37°C. The instability of TAFI has been attributed to proteolytic cleavage and a spontaneous temperature-dependent process which occurs as a consequence of conformational instability. Lowering the temperature increases the stability of TAFI from 10 minutes at 37°C to several hours at 22°C, and it is stable at 0°C. Epsilon amino caproic acid (eACA) prevents both thermal instability of TAFI and proteolytic cleavage at Arg302.

TAFI and fibrinolysis

TAFI cleaves the carboxy terminal arginine and lysine from fibrin and limits plasminogen binding as well as plasmin formation. TAFI may also directly inactivate plasmin at relatively high concentrations.

Measurement of different forms of TAFI

The instability of TAFI makes it difficult to measure the active form of the enzyme. However, a number of in-house and commercial assays have been developed to measure the different forms of TAFI. Total TAFI antigen can be measured by ELISA using mono- or polyclonal antibodies. Genotype-dependent variations in TAFI could influence such antigen assays. The Thr325Ile polymorphism (1040C/T) may lead to artefacts in TAFI antigen levels and therefore great care should be taken when evaluating TAFI antigen values. TAFI activity levels correlate to the activatable amount of pro-TAFI. The activity can

Table 1. Different forms of TAFI and their nomenclature.

<table>
<thead>
<tr>
<th>Form</th>
<th>E.C</th>
<th>Enzyme</th>
<th>Suggested new nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>zymogen</td>
<td></td>
<td>procarboxypeptidase U, R, B</td>
<td>TAFI</td>
</tr>
<tr>
<td>active form</td>
<td>3.4.17.3</td>
<td>carboxypeptidase U, R, B</td>
<td>TAFIα</td>
</tr>
<tr>
<td>inactive form</td>
<td></td>
<td>inactive carboxypeptidase U, R, B</td>
<td>TAFIβ, TAFI</td>
</tr>
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be measured following in vitro activation of pro-TAFI with the thrombin/thrombomodulin complex. TAFI activity is then determined using the substrate furyl-lactoreyl alanyl-arginine, or by measuring the release of hippuric acid from hippuril-arginine, which is detected by high-performance liquid chromatography (HPLC). Commercial kits for the determination of pro-TAFI based on chromogenic microplate assays are also available (e.g., the TAFI Actichrome activity kit from American Diagnostica, Inc., Stamford, CT, USA).

Direct determination of TAFI is problematic because of the enzyme's instability. TAFI is therefore determined indirectly by a clot lysis assay initiated with thrombin or tissue factor. Clot lysis time and the changes that occur following addition of potato tuber carboxypeptidase inhibitor (PTCI), a specific inhibitor of TAFI, indicate the level of TAFI activity and the enzyme's ability to down-regulate fibrinolysis. Good correlation has been observed between the pro-TAFI activity assays, the TAFI antigen ELISAs and the clot lysis assay with addition of PTCI.

We have recently developed the Overall Haemostatic Potential (OHP) assay. The Overall Fibrinolytic Potential (OFP) and clot lysis time derived from this assay have been used to estimate the rate of TAFI-dependent fibrinolysis in different clinical conditions. A commercial kit for the determination of TAFI and TAFII in the TAFI/TAFII complex has been developed recently by American Diagnostica, Inc. (Stamford, CT, USA), but the assay has yet to be fully characterised.

The role of TAFI in health and disease

Levels of TAFI in healthy individuals vary over a broad range. Measured as TAFI antigen, the level varies from 41% - 259%. The level of TAFI antigen does not differ between men and women. However, an increase with age has been described in females, but not in males. In contrast to the inter-individual variation, individual TAFI levels are very stable.

As the generation of TAFI is dependent on thrombin generation, clinical conditions that lead to increased or decreased thrombin levels could induce changes in the levels of the different forms of TAFI. The links between activated protein C (APC) and TAFI generation are obvious, but complex. Through TAFI and APC, the thrombin/thrombomodulin complex has a dual role in coagulation and fibrinolysis. On the one hand this complex induces APC generation, which is anticoagulant and profibrinolytic, while on the other hand it induces TAFI generation which is antifibrinolytic.

Defective thrombin generation induces a bleeding tendency in patients with inherited coagulation factor deficiencies. Recent reports describing TAFI's role in these pathways may provide an acceptable explanation for why fibrinolysis is up-regulated in patients with impaired thrombin generation. It has been shown that clots formed from FVIII, IX, X and XI deficient plasma lyse prematurely and that factor supplementation corrects this defect and at the same time increases the rate and extent of carboxypeptidase U (TAFI) activation. Addition of factor VIII restores fibrinolysis in haemophilia A plasma. While injection of recombinant FVIIa (rFVIIa) normalises overall haemostasis and TAFI dependent fibrinolysis in patients with haemophilia A, this effect is only partly replicated in vitro when rFVIIa is added to plasma with varying factor deficiencies. TAFI-dependent fibrinolysis is not completely restored.

Measurements of the different forms of TAFI have suggested that TAFI per se, could also contribute to thrombotic tendencies. Elevated TAFI antigen levels have been described as a mild risk factor for deep vein thrombosis (DVT), and increased pro-TAFI levels have been found in patients with ischaemic heart disease and stable angina. Increased TAFI antigen levels are also present in venous and coronary artery blood from patients with coronary artery disease. In addition, elevated TAFI antigen levels have been identified as a risk factor for angina pectoris. Interestingly, the incidence of the Ala 147Thr polymorphism is higher in these patients. Increased TAFI antigen levels are also associated with acute ischaemic stroke, and the level correlates with the degree of neurological deterioration. We recently found increased TAFI antigen levels in patients suffering from acute non-cardioembolic stroke. Levels decreased 60 days after the acute stroke event, but did not return to normal levels during this time.

Pro-TAFI levels are increased in hyperlipidaemic subjects with hypercholesterolaemia, while both pro-TAFI and TAFI antigen levels are increased in patients with type 2 diabetes mellitus and obesity. Elevated pro-TAFI and total TAFI antigen levels have been found in patients with nephrotic syndrome. Hypolipaemias, e.g. fluvastatin and simvastatin, significantly reduce TAFI antigen levels in patients with renal diseases. These findings may indicate an important role for TAFI in the development of atherothrombotic changes in patients with metabolic disturbances associated with hyperlipidaemia and hypercholesterolaemia. Both pro-TAFI and total TAFI antigen levels are decreased in disseminated intravascular coagulation (DIC) due to the increased turn-over of coagulation factors, platelets, fibrinogen and fibrinolytic factors associated with this condition. The further decrease in levels seen in patients with infection and organ failure suggests that TAFI may play a role in the mechanism of organ failure in DIC-associated sepsis.

TAFI levels are affected by anti-thrombotic therapy. Heparin diminishes TAFI generation, up-regulates fibrinolysis and reduces the stability of the developing clot. However, heparin does not influence fibrinolysis of stable mature clots. Argatroban and melagatran - a direct thrombin inhibitor - decrease TAFI generation and up-regulate fibrinolysis. The role TAFI plays in fibrinolysis could enhance the effects of treatment and reduce the risk of bleeding in patients undergoing therapeutic thrombolysis. PTCI (a TAFI inhibitor) significantly improves t-PA induced fibrinolysis without adverse effects in a rabbit thrombosis model.

The definitive role of TAFI in different clinical conditions has yet to be established and warrants further investigation. This, together with efforts to develop accurate and precise methods for ex vivo determination of TAFI as well as the standardisation of nomenclature, should be the main goals of TAFI research in the near future.

Further reading


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Figure 1. Simplified presentation of TAFI's role in fibrinolysis (modified from Bouna et al.).

Figure 2. Simplified presentation of thrombin/thrombomodulin's effect on coagulation and fibrinolysis (modified from Bouna et al.).