Minireview

Physiological responses to protein aggregates: Fibrinolysis, coagulation and inflammation (new roles for old factors)

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Misfolding is an inherent and potentially problematic propensity of proteins. Misfolded proteins tend to aggregate and the deposition of aggregated proteins is associated with a variety of highly debilitating diseases known as amyloidoses. Protein misfolding and aggregation is also increasingly recognized as the underlying cause of other health problems, including atherosclerosis and immunogenicity of biopharmaceuticals. This raises the question how nature deals with the removal of obsolete proteins in order to avoid their accumulation and disease. In recent years two proteases, tPA and factor XII, have been identified that specifically recognize aggregates of misfolded proteins. We here review these discoveries that have uncovered new roles for the fibrinolytic system and the contact activation system beyond haemostasis.

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1. Causes, appearances and consequences of protein misfolding

To fulfil their specific function proteins typically fold into an ensemble of closely related three-dimensional structures. This native state is however not stable and all proteins lose eventually their unique shape (i.e. become “partially unfolded” or “partially denatured”). This is an inherent property of proteins. Of particular importance in respect to findings reviewed herein is the fact that proteins unfold and show characteristics of misfolding upon direct contact with ‘denaturing’ compounds and materials. Such compounds include glass, lipids, metals and sugar polymers. Strikingly, a number of these ‘denaturing’ materials are also known to activate the contact system of blood coagulation and/or induce inflammation (see below). As a consequence misfolded proteins can lose their biological function. More problematic is the fact that misfolded proteins tend to aggregate, which is associated with the pathology of certain diseases. The propensity of proteins to aggregate varies [1,2] and many environmental conditions can accelerate the aggregation process. Examples include temperature, pH, oxidative stress, flow stress and glycation as a consequence of hyperglycemia [3]. As outlined in more detail in other reviews in this issue one specific type of aggregation is the formation of fibrils, termed amyloid [4–6]. The structure of the fibrils that are formed by the different proteins is similar [7]. They display a common structural feature: the amyloid cross-β structure, which is characterized by an organized stacking of β-sheets in the fibrils [8]. Besides the formation of fibrils, protein misfolding can result in a wide variety of aggregates with various different structural appearances, including intermediates (oligomers) of fibrils and unordered amorphous aggregates of various sizes. For more detailed information on the formation of aggregates and amyloid fibrils the reader is referred to excellent reviews [2,9]. It has been demonstrated that irrespective of the amino acid sequence oligomeric intermediates which precede amyloid fibril formation do share structural and functional properties, which are absent in native proteins [10,11].

Due to the nature or size of these aggregates the presence of cross-β structure in these protein aggregates cannot always be confirmed by X-ray diffraction, but they show other hallmarks of amyloid, such as an increased content of β-sheets, affinity for dyes such thioflavin T and Congo red. Also, amyloid fibrils are detergent and protease resistant. The term “amyloid-like” properties is often used to indicate the presence of characteristics of amyloid in other aggregates than fibrils. Thus, although aggregates are heterogeneous, they possess common non-native structural and functional properties. The exact details of these common structural aspects remain to be elucidated.

For a long time it is known that the formation of amyloid is associated with many human diseases, known as amyloidoses. Examples include Alzheimer’s disease and dialysis-related amyloidosis (for more information see [3,9] and in this issue). However,
since protein misfolding and aggregation is such a broad phenomenon it can be anticipated that misfolding and aggregation can underlie or contribute to other pathologies that are not yet identified as protein misfolding disorders. Such health problems may include atherosclerosis [12], cardiac disease [13] and auto-immune disorders, such as those associated with the use of biopharmaceuticals [14,15]. Thus, if all proteins can form hazardous species upon their unfolding, what has nature found to solve this problem? Inside cells non-native proteins are recognized by chaperones and targeted for refolding or proteolytic degradation. Although quality control in the extracellular environment has been postulated [16], it is largely unknown how accumulation of protein aggregation is prevented outside cells. In recent years, two homologous serine proteases, tPA and factor XII (FXII), were identified that recognize misfolded proteins, but not their native precursors [17,18], suggesting that the fibrinolytic system and the contact system play a role in the detection and clearance of misfolded proteins.

2. Haemostasis and the two routes of inducing coagulation

Haemostasis is the prevention of blood loss as a result of injury and the coagulation of blood is the protective mechanism. Coagulation results in the formation of a blood clot (thrombus) that contains a network of polymerized fibrin with aggregated platelets. In the classical coagulation model, described in 1964 by both Macfarlane [19] and by Davie and Ratnoff [20], coagulation is a proteolytic cascade that can be triggered by two converging enzymatic pathways, termed the extrinsic pathway and the intrinsic pathway (Fig. 1, panel a). Coagulation via the extrinsic pathway is initiated by tissue factor (TF), a transmembrane protein exposed on the injured vessel wall, that binds coagulation factor VII(a). The complex of activated factor VIIa (FVIIa) and TF activates factor X. The intrinsic pathway (all factors required for clotting are present within blood plasma) is activated in vitro when blood comes in contact with a negatively charged (anionic) surface such as glass (Fig. 1, panel b). This results in the activation of the so called contact system which consists of the zymogens factor XII, factor XI, and prekallikrein (PK) and the non-enzymatic cofactor high molecular weight kininogen (HK). Factor XII binds to the “surface” and becomes activated. Activated factor XII leads to the propagation of the intrinsic pathway by the activation of factor XI with formation of fibrin as its final consequence. Deficiencies of factors in this cascade (such as factor VIII, factor IX and factor XI) lead to bleeding disorders. This shows that the lower part of the intrinsic pathway of coagulation contributes to physiological haemostasis. Paradoxically, deficiencies in the primary contact factors factor XII, PK or HK, which activate the intrinsic pathway in vitro, are not associated with a bleeding phenotype. This suggests that these three factors do not contribute to this physiological role of the intrinsic pathway. This contradiction was explained after the identification of an alternative route of activation of factor XI: thrombin, generated by the extrinsic pathway activates FXI even in the absence of a negatively charged surface [21–24] (Fig. 1, panel a). The discovery that factor XI is activated in a factor XII-independent manner adds to our understanding of the intrinsic pathway of coagulation but still leaves factor XII without a physiological function.

Over the years, next to glass, numerous other materials were found to induce coagulation of plasma, such as the clay-like materials kaolin [25], metals, polymer sugar, dextran sulfate DXS [26–29], sulphatide [30] and ellagic acid [31]. Also more physiologically relevant “surfaces” have been shown to activate the contact system and include endothelial cell-associated glycosaminoglycans, certain nucleic acids such as extracellular RNA [32], polyphosphates [33], and also aggregated amyloid β peptide [34–36]. However, why the contact system responds to this wide variety of materials is unclear. Thus, and in addition to its paradoxical role in coagulation, the fact that a clear physiological activator of factor XII remained also unclear, made the real biological role of the contact system even more mysterious.

![Fig. 1. Classical scheme of the haemostatic systems with the new insights. The formation of a blood clot by coagulation involves the extrinsic and the intrinsic route of coagulation. (a) The extrinsic route is started upon injury by exposure of tissue factor (TF) on the injured blood vessel to VII. (b) Historically the intrinsic route was found to be induced (‘contact activated’) when blood contacts an ‘artificial’ (anionic) surface, such as a blood container, which leads to activation of factor XII into αFXIIa. However, a role of contact activation of the intrinsic system in physiological clotting has not been established and a role for the intrinsic system in the amplification of coagulation was proposed in which XI is activated by IXa (activated thrombin). (c) The contact system also leads to the formation of an inflammatory response when kallikrein (K) is formed upon cleavage of prekallikrein (PK) and when high molecular weight kininogen (HK) is cleaved into the vasoactive non-apeptide bradykinin (BK). In this process, a feedback loop results in the formation of βFXIIa, which in contrast to αFXIIa is not able to activate FXI into FXIIa. (d) The dissolution of a blood clot (fibrinolysis) is mediated by plasmin (Plm) which degrades fibrin polymers into fibrin degradation products (FDPs). Plasmin (Plm) is formed by the fibrinolytic system, when fibrin-activated tissue-type plasminogen activator (tPA) cleaves the zymogen plasminogen (Plg). tPA is also activated by misfolded protein aggregates. Moreover fibrin has properties similar to misfolded proteins (see text). Proteases are depicted in green.](image-url)
3. The kallikrein–kinin system: the formation of bradykinin

Activation of factor XII upon contact with an ‘activating surface’ results not only in the activation of the intrinsic pathway of coagulation but also in the activation of the kallikrein–kinin system by activation of prekallikrein into kallikrein, leading to an inflammatory response [37,38]. Kallikrein has several functions, but most notably, it cleaves the potent vasoactive proinflammatory nonapeptide bradykinin from high molecular weight kininogen (HK). Hence, the name kallikrein–kinin system for this pathway. Kallikrein can cleave factor XIIa(a), resulting in a positive feedback loop. A first cleavage causes surface-bound factor XII to become factor XIIa (zFXIIa), the second cleavage causes dissociation of the light–chain of factor XII, containing the protease domain. Notably, this fragment of factor XII, known as fXIIa (or factor XIII), can activate the kallikrein–kinin system, but not coagulation, in the absence of a surface [39] (Fig. 1). Thus, two very distinct enzymatic systems are triggered by active factor XII; one leading to coagulation (in vitro), the other to an inflammatory response.

Kallikrein and bradykinin have a number of roles. First, bradykinin is a potent vasoactive peptide that mediates classical parameters of inflammation, such as redness, fever, swelling, blood pressure and pain. The peptide acts through two G-protein coupled receptors, B1 and B2, that are present on a large number of cell types, but most notably endothelial cells (EC). Activation of the receptors has a role in recruitment of leukocytes and results also in the secretion of a number of proinflammatory and chemotactic cytokines [40]. Secondly, tissue-type plasminogen activator (tPA) is released by bradykinin stimulated endothelial cells [41–43]. This indicates an interplay between the kallikrein–kinin system activated by factor XII and the fibrinolytic system activated by tPA (see below) and a role for bradykinin formation in the activation of proteases necessary for remodeling of the extracellular matrix, a process necessary for homeostasis. Thirdly, the contact system can interact with the complement system: activated factor XII (fXIIa) is able to activate the complement system [44–46]. The complement system is another proteolytic cascade system in blood involved in host defense reactions, such as protection against bacterial pathogens (for review on fibrinolysis, coagulation and complement see [47]). Although most of the known roles of the kallikrein–kinin system are unrelated to coagulation, genetic deletion of both the kininogen gene and the bradykinin B2 receptor gene in mice protects against thrombosis [48,49]. But in contrast, patient studies show that levels of kallikrein and bradykinin increase after myocardial infarction, which correlates to their survival rates [50]. The kallikrein–kinin system is also shown to be neuroprotective in rat models of ischemic stroke [51,52]. Also B2 receptor knockout mice display exacerbated post-ischemic brain injury in a similar model [53]. In conclusion, the activities of the kallikrein–kinin system are diverse, but its role is likely protective under physiological conditions in order to preserve or restore homeostasis, although the system may become detrimental under pathological circumstances, as discussed in the next paragraph.

4. Involvement of the contact system in disease

Given the discovery of factor XII as inducer of coagulation in vitro, there has been special attention for its role in thrombotic disease. As discussed earlier, and in contrast to its pro-coagulant activity in vitro, the kallikrein–kinin system has protective properties in myocardial infarction and heart failure [54]. As part of an all-cause mortality study an inverse correlation was found between the levels of factor XII and the risk of cardiovascular disease [55]. Similarly, lower factor XII levels were found in patients with coronary heart disease.

Hereditary angioedema (HAE) is a life-threatening affliction caused by uncontrolled generation of bradykinin, that results in episodes of swelling in various tissues. The episodes can be triggered by allergy attacks and tissue trauma, but often the cause of an episode remains unknown. Type III HAE is caused by one of two known rare mutations in factor XII, that lead to increased factor XII activity [56]. The same pathology, HAE type I or II, is caused by deficiency or dysfunction of C1-inh, the main inhibitor of the protease activity of factor XII. The induction of blisters in the skin of HAE type I and II patients is accompanied by generation of kallikrein in the blister fluid [57].

There are many other diseases, in which the kallikrein–kinin system has been implicated, independent of its role as inducer of coagulation [58]. These pathologies include chronic renal failure [59], proliferative diabetic retinopathy [60], Alzheimer’s disease (AD) [34,61], arthritis [62–64], vasculitis [65,66], bowel disease [64], infection and septic shock [67,68], allergy [69,70], schizophrenia [71], pre-eclampsia [72], recurrent pregnancy loss [73–76] and cancer [77] and the majority of them have an inflammatory component. Whether activation in all these conditions is protective or detrimental remains to be established, but an intriguing question that arises is why and how the contact system becomes activated, especially since the presence of contact-activating surfaces has not been reported in all these conditions. Once again, the identification of physiological activators is required not only to understand the activation of factor XII in vivo but also to explain why activation of the kallikrein–kinin system is not paralleled by activation of the coagulation system in vivo.

5. The fibrinolytic system

Fibrinolysis is the process that results in the degradation of fibrin polymers during the removal of blood clots. This process contributes to wound healing and haemostasis. Similar to the contact system, it is a proteolytic mechanism that results in the formation of one main effector enzyme, plasmin, which cleaves fibrin (Fig. 1, panel d). Plasmin is formed via activation of its liver–produced zymogen plasminogen by either tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA). tPA, which is structurally homologous to factor XII (see below), is released by activated endothelial cells [41–43]. This indicates an interplay between the kallikrein–kinin system activated by factor XII and the fibrinolytic system activated by tPA (see below) and a role for bradykinin formation in the activation of proteases necessary for remodeling of the extracellular matrix, a process necessary for homeostasis. Fibrinolytic systems are triggered by active factor XII; one leading to coagulation (in vitro), the other to an inflammatory response.

6. Additional activators and roles for tPA in various processes other than fibrinolysis

Due to its discovery as initiator of fibrinolysis and its activation by fibrin polymers, tPA and the fibrinolytic system are associated with the dissolution of blood clots. However, it has been demonstrated over the years that (1) tPA can be activated by many proteins, (2) plasmin has many substrates other than fibrin and (3) the ‘fibrinolytic’ system has biological functions independent from fibrin and separate from its role in blood clot lysis. Examples of proteins other than fibrin that activate tPA include, extracellular matrix proteins [79], thrombospondin [80], histidine-proline-rich glycophorin [81], maspin [82], actin [83,84], endostatin [85], and others [86–89].
glycated proteins [86], proteins on the surface of various pathogens (reviewed in [87]), amyloid-β peptide associated with Alzheimer’s disease [84,88,89], prion protein of Creutzfeldt-Jakob’s disease [90], and denatured proteins [91–95]. In addition to polymerized fibrin in blood clots, also denatured or aggregated forms of its predecessor, fibrinogen, can activate tPA [95,96]. Intriguingly, many activators of tPA, including fibrin fragments have been demonstrated to inhibit angiogenesis and may be applicable to treat cancer [97,98]. Some potential sites in fibrin have been implicated in the activation of tPA by fibrin [99], but the exact mechanism of activation by fibrin and by all these other activators remain to be elucidated.

Plasmin has a broad substrate specificity and degrades other extracellular matrix proteins such as laminin. In addition, plasmin activates pro-collagenases, allowing degradation of collagen, and plasmin cleaves prohormones, for example latent TGF-β. Given their broad specificity it seems unlikely that the role of tPA and plasmin is limited to the dissolution of fibrin in blood clots. Indeed, both tPA and plasmin have been demonstrated to play a role in several processes [100], that include tissue remodeling and most notably, neuronal cell migration and neuronal function, such as long term potentiation [101].

Studies indicate that tPA modulates the deposition of amyloid-β peptide that is associated with Alzheimer’s disease [84,102–104]. Plasmin degrades amyloid-β aggregates in vitro [84,104] and amyloid-β injected into the brain is not removed in tPA-deficient mice [103]. Based on these findings the effects of a PAI-1 inhibitor was studied in a mouse model of Alzheimer’s disease [102]. This compound, which sustains plasmin activity, enhanced amyloid-β clearance. Thus activation of fibrinolysis may be a novel disease modifying treatment for amyloidosis. However, tPA can also be detrimental. In animal models it has been shown that tPA is an essential element in the induction of excitotoxic injury [105]. As with the contact system, the fibrinolytic system is implicated in a large number of pathologies, including amyloidosis, cancer, infections, atherosclerosis and auto-immune diseases. Another intriguing overlap between the activation of the contact system by ‘surfaces’ and the activation of the fibrinolytic system is that plasminogen plays a central role in the inflammatory response to biomaterials [106].

Taken together, this leads to the central questions: (1) what is the common denominator among all these different activators of factor XII and tPA and (2) how do these factors bind to their various activators. Recent publications have gained insight into these two questions.

7. Misfolded proteins comprise similar features that activate tPA and factor XII

As described above and in detail elsewhere in this issue and other reviews proteins can aggregate in multiple ways, resulting in aggregates with heterogeneous appearances. Also, not all unfolded proteins will aggregate, be toxic or have an amyloid-like structure. Aggregates can be amorphous, form oligomers or fibrils. Within these varieties, common structural and functional properties have been found, including the propensity to induce cellular toxicity [10,11]. The details of the structural commonalities in oligomers and amorphous aggregates remain to be elucidated, as well as the exact mechanism(s) responsible for the cellular toxicity. The current evidence implicates so-called soluble oligomers as the most toxic species (see other reviews in this issue). Common structural characteristics of misfolding and subsequent amyloid fibril formation include the increase in β-sheet content and folding into the cross-β structure. Such features are directly measured using circular dichroism (for β-sheet content) and X-ray fibre diffraction (cross-β structure). Alternatively certain dyes, most notably thioflavin T (ThT), can be used. Its binding induces fluorescence and is indicative for structural changes reminiscent to amyloid cross-β structure. The exact mode of binding of thioflavin T remains however to be established, although recent publications indicate that thioflavin T binds in cavities of the amyloid fibrils [107,108]. Native proteins do generally not comprise properties common to amyloid fibrils or their precursors, including affinity for thioflavin T and presence of cross-β structure.

Based on the fact that tPA was activated by numerous proteins, including denatured proteins and amyloid-β (see above) we hypothesized that tPA is activated by common features to misfolded proteins and demonstrated that tPA is indeed a cross-β structure receptor [18]. We demonstrated that activation of tPA correlated with the presence of cross-β structure and is independent of the amino acid sequence of the underlying protein (Fig. 2a). Moreover, the binding of tPA to misfolded proteins comprising cross-β structure could be inhibited with Congo red (Bouma, unpublished). Subsequently we established that the homologous protease, factor XII, is activated by misfolded proteins as well and that this leads to kallikrein formation but not to activation of factor XI into Xla [17] (Fig. 2b–d). Activation of factor XII resulting in kallikrein formation was only observed with misfolded protein aggregates but not with native, monomeric species nor with amyloid fibrils. We also demonstrated activation of both the contact system and the fibrinolytic system in vivo in patients with systemic amyloidosis [17,109]. In line with the results in vitro, activation of the kallikrein system was seen in patients with amyloidosis, but no activation of factor XI was notable [17]. These findings uncovered a possible role for tPA and factor XII in modulating protein misfolding and protein misfolding diseases.

8. Surface-induced conformational changes reminiscent of misfolding underly the mechanism of activation of the contact system

In our efforts to elucidate the physiological mechanism of activation of factor XII we examined the role of protein misfolding in classical activation of factor XII by surfaces [17]. For a long time it is known that the contact system is activated by ‘surfaces’ and that this results in activation of factor XI and prekallikrein. It is also well known that proteins adsorb to surfaces, such as glass, polymers and also phospholipids. The adsorption of proteins depends on the molecular weight and with time a low molecular weight protein is displaced by higher molecular weight proteins. This effect is known as the Wromen effect and was established with blood plasma [110]. Many publications have demonstrated that protein adsorption can result in structural changes and denaturation of these adsorbed proteins [111,112]. One example includes fibrinogen, the precursor protein of fibrin in blood clots, adsorbed to glass [113]. Recent studies have now also directly demonstrated protein adsorption to certain materials, including phospholipids [114,115] and surfaces, like mica [116], with the formation of the structural features common to misfolded proteins and reminiscent of amyloid. In our recent work on the activation of the contact system we showed that the activation of prekallikrein by factor XII could not be induced by a ‘surface’ alone, but was completely dependent on the presence of sufficient amounts of protein [17] (Fig. 2e). We also demonstrated that the surfaces that induce factor XII activation through a cofactor protein also induced conformational changes in these cofactor proteins that likely underly the activation of factor XII (Fig. 2f). Whereas activation of the kallikrein system required addition of proteins (to be denatured by the surface) activation of factor XI was seen in the absence of proteins (Fig. 2d). Hence activation of factor XI can be triggered by direct contact of
factor XII to the surface. The mechanism is still unknown, but may involve conformational changes and autoactivation of factor XII upon binding to the surface. Such changes have been observed [117]. A thorough analysis of the literature revealed that in the past factor XII activation studies were always performed in the presence of protein in the assay buffer, which explains previously observed kallikrein activation. Recently it was concluded that the paradigm that negatively charged surfaces activate the contact system required substantial revision as also positively charged materials can activate factor XII and that the protein composition of the fluid plays an important role [118]. Our work has now demonstrated that the real trigger for activation of factor XII for formation of kallikrein is the change in protein conformation, i.e. induction of unfolding and misfolding, due to contact of a native protein with a non-compatible surface. In this view, the activation of prekallikrein by factor XII observed in all previous studies should be reconsidered. In these studies the activator of factor XII may in fact be a protein unfolding (misfolding) inducing agent. Taken together,
our work has changed the view on the activation of factor XII and revealed insight into the physiological role of the contact system (see below). These findings are of particular importance for the safety of biomaterials, such as stents, and for the manufacturing, packaging, and storage of biopharmaceuticals [14,15,119]. Hence tPA and factor XII can be applied as sensitive measurement methods of biomaterial hemocompatibility and the quality of biopharmaceuticals [15,120]. This may help to decrease the adverse inflammatory effects of biomaterials, such as hemodialysis systems or stents, and the immunogenicity of biopharmaceuticals.

9. The fibronectin type I domain (‘finger domain’) – a motif that binds misfolded proteins

How do factor XII and tPA recognize their activators, i.e. misfolded proteins. These proteases are quite homologous and comprise several distinct motifs (Fig. 3). Using deletion mutants and recombinantly produced domains we recently mapped the binding site to the fibronectin type I (or ‘finger’) domain [121]. We also found that similar domains in yet another homologous protein, hepatocyte growth factor activator (HGFA) and in the extracellular matrix protein fibronectin bind protein aggregates. We demonstrated that isolated fibronectin type I domains can inhibit platelet aggregation induced by misfolded protein aggregates, which may offer new therapeutic strategies to treat diseases associated with protein misfolding. The same domains were previously shown to mediate the interaction of tPA with fibrinogen [78,122] and fibronectin with fibrin [123]. This suggests that the interaction of tPA with misfolded proteins and fibrin may result from common structural features. Hence, does fibrin has amyloid-like properties?

10. What about misfolding properties in fibrin?

Some specific amino acid sequences in fibrin have been identified to induce tPA activation [124]. However, the evidence has been gathered with the use of synthetic peptides. We showed, at least for one peptide (amino acid 148–160 of the alpha-chain of fibrinogen), that, while the crystal structure predicts the formation of α-helix, these peptides form cross-β structure in vitro [18]. At present it is widely accepted that the structural changes leading to the exposure of sites thought to bind t-PA and/or plasminogen remain to be demonstrated [125]. We studied structural properties of fibrin and found that fibrin has structural properties reminiscent of amyloid (Bouma, manuscript submitted). Thrombin-induced polymerized fibrin binds the amyloid dyes, Congo red and thioflavin T. Fibrin also binds serum amyloid P (SAP), a protein used to diagnose amyloidosis also in vivo [126]. Finally we demonstrated the presence of cross-β structure using X-ray fibre diffraction analysis. Our results fit with early data on the structural changes that are associated with the conversion of fibrinogen into fibrin that showed that fibrin formation is accompanied by a general increase in β-sheet formation of hydrogen bonds between adjacent fibrin molecules [127]. This type of interaction within the fibrin meshwork is similar to that underlying the cross-β structure in amyloid, suggesting that these cross-β structures are formed during fibrin polymerization. Hence, this common structural element, which can be formed by a myriad of proteins including fibrin, triggers fibrinolysis, contributing to the removal of obsolete proteins, such as fibrin in blood clots, and potentially also all other proteins. Just recently, it was shown that fibrin also activates factor XII [128], supporting the idea that a common structural element is present in fibrin and protein aggregates to activate both tPA and factor XII.

11. The Crossbeta pathway: new roles for two old factors

Together, new roles have been identified for two old factors: factor XII and tPA. We have shown that both the contact system and the fibrinolytic system react to misfolded proteins of various origin. Activation of factor XII by misfolded proteins leads to kallikrein and BK formation but not to factor XIa generation. This implies that in this situation the intrinsic pathway of coagulation is not triggered but that instead a fast vasoactive and proinflammatory response is the result. The fibrinolytic system is also activated because BK induces the release of tPA from endothelial cells while kallikrein activates both urokinase-type plasminogen activator and plasminogen. Earlier, we reported that tPA-dependent plasmin for-

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**Fig. 3.** Schematic representation of the structure of factor XII, tPA, HGFA and fibronectin. Domain architecture of tPA, factor XII, hepatocyte growth factor activator (HGFA) and fibronectin. F, fibronectin type I (‘finger’) domain; E, EGF-like domain; II, fibronectin type II domain; III, fibronectin type III domain; Pr, proline-rich region; K, Kringle; P, protease domain.
mation is stimulated by misfolded proteins. Plasmin not only degrades fibrin clots but also plays a role in inflammation and wound repair. Evidence for the generation of kallikrein but not FXIa and activation of the fibrinolytic system was obtained in patients with systemic amyloidosis. Activation of factor XII resulting in kallikrein formation was only observed with misfolded protein aggregates but not with native, monomeric species or amyloid fibrils. The protein aggregates resemble prefibrillar, oligomeric species that are held responsible for the toxicity seen in protein misfolding disease. We therefore propose that the contact system and the fibrinolytic system contribute under normal conditions to the detection and removal of otherwise potential harmful protein aggregates, before they become protease resistant amyloid fibrils. As mentioned above activation of the contact system was observed in a wide variety of diseases i.e. diabetic retinopathy, lactic acidosis and inflammatory bowel disease. It is tempting to speculate that in these conditions for instance changes in pH have led to the formation of misfolded proteins and as a consequence activation of factor XII. Since misfolded proteins can be formed under a variety of conditions (e.g. pH, temperature, oxidative stress, flow stress, glycation) and by a large number of “denaturing” compounds and materials (e.g. glass, lipids, endothelial associated glycosaminoglycans, RNA) it is anticipated that protein aggregation is much more widespread than currently known. For example, protein aggregation may underly pathological conditions with at present unknown etiology in which factor XII activation has been implicated, i.e. auto-immune disease such as those associated with the use of biopharmaceuticals or the anaphylactic reactions observed with certain preparations of heparin that were contaminated with oversulfated chondroitin sulfate [129,130].

Unfolding of proteins is a fact of nature. We propose that with the identification of tPA and factor XII we have uncovered an ancient pathway for the clearance of unfolded proteins that facilitates protein homeostasis and prevents against protein misfolding diseases. This fits well with the suggested protective role for factor XII [55]. The complement system and certain scavenger receptors, like the receptor for advanced glycation end-products RAGE XII [55], CD36 [133] and LRP [134] that were also shown to bind like the receptor for advanced glycation end-products RAGE. The complement system and certain scavenger receptors, protein homeostasis and prevents against protein misfolding diseases. FEBS Lett., doi:10.1016/j.febslet.2009.05.040.

Conflict of interest

The authors are employees and/or hold shares in Crossbeta Biosciences, a spin-off company of the Utrecht University and University Medical Center Utrecht.

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