Regular Article

Regulatory mechanisms of the plasma contact system

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ABSTRACT

The plasma contact system is a proinflammatory and procoagulant protease system. The biochemistry of the system is well established, however, its biological functions are just beginning to emerge. Here, we provide a condensed overview on mechanisms involved in activation and regulation of the contact system. These recent findings will help us to better understand the role of the enigmatic system for health and disease.

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Introduction

The contact system consists of the two proenzymes factor XII (FXII) and plasma prekallikrein, as well as the non-enzymatic procofactor high-molecular-weight-kininogen (HK) that locally assemble and become activated on negatively charged surfaces [1]. Contact system activation initiates fibrin formation via the intrinsic pathway of coagulation. Remarkably, deficiencies in the contact factors lead to prolonged clotting times in vitro, but do not cause bleeding complications in vivo. Additionally, the contact system forms the proinflammatory peptide-hormone bradykinin (BK) via plasma kallikrein (PK)-mediated HK cleavage [2]. BK increases vascular permeability and regulates inflammatory cells [3]. The physiological function of the contact system in vivo has remained enigmatic. However, animal studies suggest a potential pivotal role of the system for thrombosis. As the contact system is dispensable for hemostatic mechanisms, targeting the contact system offers the exciting perspective to treat thrombotic disease without therapy-associated bleeding complications [4].

Contact activation

Identification of the FXII surface-binding site that induces activation of the zymogen has proven to be a challenging task for decades [5]. So far, no FXII crystal structure is available. Studies based on FXII deletion mutants and blocking-antibodies indicated that the Fibronectin type I domain [6], the Fibronectin type II domain [7], the proline-rich region [8], as well as a positively charged residues in the N-terminus of FXII [9] contribute FXII surface binding. On negatively charged surfaces, FXII activation proceeds in two alternative mechanisms: 1) FXII binds to a surface, attains limited proteolytic activity most likely because of conformational changes [10], and undergoes autoactivation by limited proteolysis [11, 2] Alternatively, PK cleaves surface-bound FXII zymogen. PK is much more capable in cleaving FXII zymogen compared to FXIla [12]. In both cases, a single cleavage of the peptide bond R353-V354 activates FXII to FXIla. The importance of this primary cleavage site is illustrated by the observation that a single point mutation at residue R353 completely silences FXII zymogen activation [13]. The resulting product of this initial cleavage, FXIla, is a two-chain protein composed of a 353 amino acid heavy chain and a 243 amino acid light chain. Both chains remain connected by a disulfide bridge, spanning cysteines 340 and 467. Simultaneously, the catalytic site becomes exposed, resulting in α-FXIIa.

What are the structural requirements for a surface that induces FXII autoactivation or PK-mediated zymogen activation? High molecular weight dextran sulphate (Mr 500,000 and 50,000) initiates FXII activation in plasma, supports FXII autoactivation and enhances FXII susceptibility for cleavage by PK. In contrast, low molecular weight dextran sulphate (Mr 15,000 and 5,000) neither induces contact system activation in plasma, nor supports autoactivation of FXII, although these agents enhance the rate of activation of FXII by PK in purified system [14]. Surface binding does not only initiate FXII autoactivation. PK-mediated cleavage of bound FXII is facilitated by a conformational change in FXII [15]. Further FXIIa cleavages at R343-L344 and R334-N335 release the protease domain from the surface-binding heavy chain. The resulting 30 kDa product, β-FXIIa (also known as FXII fragment or FXIIf) [16] and has the capacity to activate PK [17], but not FXI, in contrast to α-FXIIa [18]. This may be explained by the finding that the heavy chain of α-FXIIa contains a binding site for the fourth apple domain of Factor XI (FXI) [19] but not for PK [20, 21]. Besides activating FXI and PK, β-FXIIa can also induce Factor VII activation [22] and triggers complement activation [23] and fibrinolysis [24, 25].
Contact system inactivation

C1 esterase inhibitor (C1INH) is the predominant plasma inactivator of both α-FXIIa and β-FXIIa [26]. C1INH belongs to the serpin family and inactivates enzymes by acting as a 'suicide' substrate that irreversibly binds to the active sites following cleavage. On procoagulant artificial surfaces such as kaolin, the rate of inactivation of α-FXIIa by C1INH is reduced [6]. C1INH also inactivates PK [27] and functional C1INH deficiency results in excessive bradykinin formation and angioedema [28]. In similar fashion, gain of function mutations in FXII result in the same phenotype [29]. Interestingly, C1INH deficiency is not overtly associated with thrombotic episodes. This indicates that the contact system can be activated in a coagulation-independent manner during these attacks.

Contact activators of Factor XII

Various artificial agents have been reported to initiate FXII activation (reviewed in [5]). Mostly, activation of both the intrinsic coagulation and the kallikrein-kinin system occurs simultaneously, as is the case during FXII activation triggered by kaolin or ellagic acid. Consistently, negatively charged compounds have been identified that activate FXII in vivo: Extracellular RNA is prothrombotic in a FXII-dependent manner in mouse models for thrombosis, demonstrated by the thromboprotective effects of RNAse (that degrades RNA) [30]. Similarly, collagen, a previously known activator of FXII in vitro [31], has been warranted as a candidate activator of FXII during thrombosis in vivo [32]. Finally, a recent report identified that the previously characterized in vitro FXII activator polyphosphate [32], also acts as the FXII activator on activated platelets in vivo. Polyphosphate is an inorganic polymer composed of long stretches of phosphate units that is released from platelet dense granules and drives the formation of occlusive intravascular thrombi in a mouse model for this disease [34]. Moreover, this compound is capable of activating inflammatory responses via the kallikrein-kinin system. FXII-deficient mice are protected against thrombosis by their inability to form stable thrombi [35]. Hence, polyphosphate-driven FXII activation mostly contributes to fibrin formation during the propagation phase of pathological thrombus formation, rather than during the initiating events that surround vascular injury (Fig. 1). Platelets from patients with Hermansky-Pudlak syndrome that lack dense granules are defective in contact-system driven coagulation [34]. Hence, it is attractive to think that the absence of released polyphosphate causes this defect but other dense granule components may contribute indirectly. So far, the mechanism of polyphosphate synthesis has not been elucidated in murine or in human cells and there are no reports of specific polyphosphate deficiency in these species. Thus, an appraisal of the hemostatic changes that are related to polyphosphate-deficiency would be of interest to confirm the primary identification of a pathologic interaction between polyphosphate and FXII.

Recently, there has been much attention for FXII activators that are specifically involved in thrombotic disease by triggering coagulation. Intriguingly, several activators of FXII only trigger kallikrein-kinin system activation, but do not evoke a coagulation response. For instance, lipopolysaccharide (LPS) activates FXII [36] and administration of LPS to baboons [37] or mice [38] contributes to hypotension in a FXII-dependent manner, but does not trigger intravascular coagulation. Similarly, aggregated amyloid β peptide triggers FXII activation [39] and activation of the contact system was reported in Alzheimer's disease [40]. However, also other sources of damaged and aggregated proteins can trigger FXII activation with unilateral activation of the kallikrein-kinin system [41]. FXII-driven kallikrein-kinin system activation has been implicated in allergic hypersensitivity and anaphylactoid reactions, amongst others to biopharmaceutical products such as heparin. These reactions may be triggered by highly sulphated contaminants in therapeutic heparin preparations after administration [42], but normally are not triggered by therapeutic heparin preparations. Pharmaceutical heparin preparations are usually infused intravenously or subcutaneously. Bradykinin that may be generated by heparin infusions is degraded during the first lung passage before reaching blood pressure-regulating pre-capillary vascular beds. However, when allergen-activated mast cells release heparin, it triggers FXII to activate, resulting in activation of the kallikrein-kinin system but not in FXI activation [43]. These findings suggest that activation of the kallikrein-kinin by mast-cell released heparin system may be related to the function of these cells, both in its physiological protective role, as well as in mast-cell related diseases. Another important question that remains is, what the regulatory mechanisms are that underlie the varying responses of coagulation FXII to surfaces. Studies with highly charged fractionated dextran derivatives have elegantly demonstrated that autoactivation responses of FXII differ with increase in molecular size; whereas high Mr chains drive FXII to form α-FXIIa only, β-FXIIa is rapidly formed on smaller chains with the same charge density [44]. Whether differences in efficacy of β-FXIIa formation in response to various agonists underly the regulation of FXII will require further investigation.

**Fig. 1. Role of FXII in coagulation.** Haemostasis: Thrombin (FII) formation at sites of lesions is predominantly due to tissue factor (TF) exposed in the subendothelial matrix. TF in complex with factor VII (FVII) initiates thrombin formation, which promotes fibrin formation and platelet activation. The contribution of factor XII (FXII) for hemostasis is minor. Thrombosis: Additional fibrin forming activity is necessary to form a thrombus when TF/FVII-driven coagulation is blocked by tissue factor pathway-inhibitor (TFPI). FXII is activated by platelet polyphosphate on activated platelet surfaces contributes to thrombin generation and additional platelet activation, propagating thrombus growth. Accordingly, FXII- as well as factor XI (FXI)-deficiency severely impairs thrombus formation but have no function for haemostasis.
Novel findings

FXII-deficient mice are protected in models of arterial thrombosis, pulmonary embolism and stroke [35,45]. Recent findings have extended these findings by demonstrating that pharmacological contact system inhibitors, such as recombinant infestin4, fused to albumin can confer similar protection in these models [46]. Moreover, blocking expression of contact system factors with antisense oligonucleotides provides protection in models for arterial and venous thrombosis [47,48]. In clinical studies on human thrombosis, determinations of FXII antigen and levels of FXIIa activity have provided contradictory data [49–52]. Moreover, there is no clear evidence to suggest that deficiency in contact factor protects from thrombosis. However, case reports as well as clinical studies have indicated the occurrence of thromboembolic events in these persons [53,54]. In conclusion, the overall picture of the contribution of the contact system to health and disease remains elusive. suggesting that the acute prothrombotic role of FXII that has been established in animal models may be counteracted by a long-term protective physiological role of the contact system. Since FXII-deficient mice are similarly protected as FXI-deficient animals, FXII-driven fibrin formation proceeds via the FXIIa substrate factor XI. Indeed, an antibody that disturbs FXI activation by FXIIa protects in murine and primate models of thrombosis [55]. Interestingly, the same paper indicates that FXII-deficient mice are protected against thrombosis to a larger extent than their FXI-deficient counterparts, suggesting that FXII also contributes to thrombosis in a FXI-independent manner. Indeed, FXIIa may activate FVIII during thrombosis [22] or could activate platelet procoagulant-activated receptor 3 [56]. However, it should be noted that this receptor is specifically present on murine platelets. Furthermore, the plasma protein histidine-rich glycoprotein (HRG) binds to FXIIa and inhibits FXII autoactivation in a zinc-dependent manner, which is proposed to occur in the vicinity of a developing thrombus [57]. Additionally, FXIIa directly alters clot structure, leading to a denser, more rigid organization of fibrin polymers [58]. Since such differences in clot structure density have previously been related to an elevated risk on thrombotic disease [59], FXIIa may modulate thrombosis by influencing fibrin structure.

Conflict of interest statement

The authors have no relevant financial interests to declare.

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