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Competitive-protein adsorption in contact activation of blood factor XII ☆, ☆☆

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Abstract

Contact activation of blood factor XII (FXII, Hageman factor) is moderated by the protein composition of the fluid phase in which FXII is dissolved. Solution yield of FXIIa arising from FXII contact with hydrophilic activating particles (fully water-wettable glass) suspended in a protein cocktail is shown to be significantly greater than that obtained under corresponding activation conditions in buffer solutions containing only FXII. By contrast, solution yield of FXIIa arising from FXII contact with hydrophobic particles (silanized glass) suspended in protein cocktail is sharply lower than that obtained in buffer. This confirms that contact activation is not specific to anionic hydrophilic surfaces as proposed by the accepted biochemistry of surface activation. Rather, contact activation in the presence of proteins unrelated to the plasma coagulation cascade leads to *an apparent specificity* for hydrophilic surfaces that is actually due to a relative diminution of activation at hydrophobic surfaces and an enhancement at hydrophilic surfaces. Furthermore, the rate of FXIIa accumulation in whole-plasma and buffer solution is found to decrease with time in the continuous presence of activating surfaces, leading to a steady-state FXIIa yield dependent on the initial FXII solution concentration for both hydrophilic and hydrophobic procoagulant particles suspended in either plasma, protein cocktail, or buffer. These results strongly suggest that activation competes with an autoinhibition reaction in which FXIIa itself inhibits FXII→FXIIa. Experimental results are modeled using a reaction scheme invoking FXII activation and autoinhibition linked to protein adsorption to procoagulant surfaces, where FXII activation is presumed to proceed by either autoactivation ($\text{FXII} \xrightarrow{\text{surface}} \text{FXIIa}$) and autohydrolysis ($\text{FXII} \xrightarrow{\text{FXIIa}} 2\text{FXIIa}$) in buffer solution or autoactivation and reciprocal activation (kallikrein-mediated hydrolysis) in plasma. FXII adsorption competition with other proteins in the fluid phase is proposed to affect the balance of activation and autoinhibition, leading to the observed moderation of FXIIa yield.

Keywords: Blood coagulation; FXII; Hageman factor; Contact activation; Autoactivation; Protein adsorption

1. Introduction

Development of truly hemocompatible materials for a wide variety of cardiovascular devices remains a substantially unrealized objective of biomaterials science [1–3]. Even after decades of focused research, thrombosis remains *the significant barrier* to development and implementation of advanced blood-contacting medical devices [4,5]. Clearly, an improved understanding of the events leading to thrombus formation on biomaterials is needed that will define new bioengineering routes to hemocompatibility. An approach we have taken has been to simplify the problem down to the minimum essential unit of study [6–12].

☆A Contribution from the Hematology at Biomaterial Interfaces Research Group, The Pennsylvania State University.

☆☆*Impact statement*: This work strongly suggests that the traditional understanding of hemocompatibility must be revised to accommodate an important role for competitive-protein adsorption in contact activation of blood coagulation that is unrelated to enzymatic inhibition or allosteric regulation.

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This strategy eliminates blood flow as it occurs in natural or synthetic blood conduits and further divides blood into cellular and humoral (plasma) categories.

Research into plasma-phase coagulation has focused on surface interactions with biomaterials that activate the so-called plasma coagulation cascade. This cascade is an interconnected series of self-amplifying, zymogen-enzyme conversions that penultimately produces thrombin (FIIa), a powerful serine protease. In the final step of the plasma coagulation cascade, FIIa hydrolyses fibrinogen into fibrin units that oligomerize into a fine mesh which causes plasma to gel or clot. The plasma-coagulation cascade is formally divided into two branches, the intrinsic and extrinsic pathways, that can be separately potentiated but merge into a common pathway leading to thrombin release. The extrinsic pathway is responsible for hemostatic control and response to vascular injury. The normal physiological function of the intrinsic pathway is not well understood [13,14], but has been implicated as an important cause for poor hemocompatibility of cadiovascular biomaterials because it is triggered by contacting blood with artificial materials [15–20].

The initiating step of the intrinsic pathway is surface-contact activation of the blood zymogen FXII (Hageman factor) into an active enzyme form FXIIa ($\text{FXII} \xrightarrow{\text{surface}} \text{FXIIa}$, also termed autoactivation in the hematology literature [13]). FXII and FXIIa are central members of a self-amplifying activation complex involving the allosteric proteins prekallikrein (PK, Fletcher factor), high-molecular-weight kininogen (HMWK, Williams-Fitzgerald factor), and FXI (thromboplastin antecedent) [13]. FXII activation is thought to occur by contact or binding with material surfaces [13,20]. The exact nature of the FXII contact/binding step is a matter of continued investigation in biomaterials surface science [7,10], but the consensus opinion has long been that autoactivation occurs most efficiently when blood contacts "anionic" [21–23] or "hydrophilic" (water wettable) procoagulants [6–8,10]. Specificity for anionic hydrophilic surfaces is presumed to be due to a chemically specific binding event between FXII, HMWK, and surface-resident negative charges. A complex formed from PK, Factor XI, and surface-bound HMWK is thought to bring all factors and co-factors into reactive proximity [19,24].

We have pursued studies of whole-plasma coagulation seeking to resolve detailed relationships among biomaterial surface chemistry, energy, and the propensity to activate the *intact* plasma coagulation cascade [6–12]. Results of these 'holistic' studies [11] have motivated us to serially simplify the focus of investigation from whole plasma, to a group of coagulation proteins, to finally purified enzymes of the cascade as a means of comparing activation of the pieces to activation of the whole [10,11,25]. In very brief summary, we found that potentiation of the intrinsic pathway in vitro leads to *bolus* release of thrombin (FIIa) in concentration proportion to the intensity of activation as measured by procoagulant surface area or surface energy (hydrophilicity, water wettability). Bolus production of FIIa suggests that the cascade somehow 'turns off' in the continuous presence of activating surfaces very soon after initial activation. Otherwise, continuous production of FIIa would be anticipated [10], not the punctuated release of a bolus inferred from experimental results. Autoactivation appears to be a likely point of control [21], leading us to further investigate how surface chemistry affects the $\text{FXII} \xrightarrow{\text{surface}} \text{FXIIa}$ reaction isolated from all other proteins. In so doing, we found the contrast between surface activation of whole plasma coagulation and FXII activation in pure-buffer solution nothing less than startling.

First, we were surprised to learn that FXII activation *in neat-buffer solution* (no proteins other than FXII) is not specific for anionic hydrophilic procoagulants as thought previously [25]. In fact, slightly greater FXIIa yield was measured at hydrophobic (poorly water-wettable, silanized-glass) surfaces than at an equivalent surface area of hydrophilic (fully water-wettable, clean glass) procoagulant surfaces. Second, we found that FXII activation in neat-buffer solution was effectively instantaneous upon contact with either hydrophilic or hydrophobic procoagulant type. Third, in sharp contrast to this second finding, we discovered that activation rate-and-yield were significantly attenuated at hydrophobic surfaces *in the presence of plasma proteins*, but not at hydrophilic surfaces. Finally, we found that auto-hydrolysis ($\text{FXII} \xrightarrow{\text{FXIIa}} 2\text{FXIIa}$) was a facile reaction in neat-buffer solution but insignificant in plasma [11,26]. Needless to say perhaps, these new findings are quite contrary to expectations based on the traditional understanding of autoactivation biochemistry briefly outlined above.

Our collective interpretation of this new experimental evidence is that FXII activation in plasma leads to *an apparent specificity* for hydrophilic surfaces that is actually due to a relative diminution of the FXII→FXIIa reaction at hydrophobic surfaces, not to a real specificity for anionic/hydrophilic surfaces [25]. We speculated that depression of activation rate-and-yield at hydrophobic surfaces immersed in plasma results from a protein-adsorption competition between FXII and a host of other proteins that substantially reduced frequency of FXII–surface interactions which, in turn, reduced efficiency of activation at hydrophobic procoagulants. Presumably, little-or-no such protein-adsorption competition occurs at hydrophilic surfaces because fully water-wettable surfaces adsorb little-or-no protein [7,27–31]. This work confirms these speculations and elaborates our interpretation into a scheme that incorporates competitive-protein adsorption into a model of contact activation of blood coagulation.

2. Methods and materials

2.1. Plasma and coagulation proteins

Citrated human platelet poor plasma (PPP) was prepared from outdated (within 2 days of expiration) lots obtained from the M.S. Hershey Medical Center Blood Bank. This work was performed with a