Procoagulant Efficiency of Activated Hageman Factor (FXIIa) on Solid Substrates

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Statement of Purpose: Despite widespread use of blood-contacting medical devices there is little understanding of the surface-mediated molecular events that occur when a biomaterial interacts with blood plasma, an important consideration to develop hemocompatible materials. Previous studies suggest that the intrinsic cascade of coagulation is initiated by binding of FXII to a negatively charged surface, and that activated FXIIa undergoes repeated adsorption-desorption events vicinal to the interface in order to interact with other proteins to form the contact activation complex for self-amplification and propagation of the cascade [1]. Moreover, specific interactions between the negative surface charge and FXIIa is believed to be essential for the procoagulant activity of the enzyme. In this study, human FXIIa was pre-adsorbed onto model surfaces of different surface energy and the procoagulation efficiency was investigated in vitro to test whether repeated adsorption-desorption of FXIIa is essential for material-induced coagulation.

Methods: Silanized glass beads (500 um dia) with terminal methyl (OTS), amine (APS) and carboxyl (COOH) groups were prepared as per previous procedures [2]. A known surface area of bead activators was incubated in protein solutions (FXIIa or BSA) for 2 hrs and washed thereafter to remove unbound protein. To prevent desorption of weakly-bound proteins from hydrophilic COOH and APS surfaces, proteins were chemically linked by activation and coupling using carbodiimide and glutaraldehyde, respectively. Solution depletion measures using the NanoOrange® Protein Quantitation kit (Molecular Probes, OR) were used to determine the amount of bound protein. The procoagulant efficiency of FXIIa conjugates was assayed in recalcified FXII-deficient platelet poor plasma (dPPP, 50% dilution with PBS) contained in a 5ml polystyrene (PS) vial by recording the coagulation time (CT) corresponding to varying doses of these protein-coated beads [2].

Results: Three different silanized glass-beads designated as OTS, APS and COOH, with increasing hydrophilicity were used as solid substrates. Water contact angle was used to confirm the surface energetics and the procoagulant efficiency determined from surface area titration exhibited increased "catalytic efficiency" with increasing hydrophilicity (summarized in Table 1), consistent with previous results [2].

Table 1: Sessile-drop water contact angles and catalytic efficiency of solid procoagulants

Procoagulant Surface	Contact Angle	Catalytic efficiency (K_1)
OTS: hydrophobic	~ 110°	0.012 ± .006
APS: partially-hydrophilic	~ 60°	0.037 ± .013
COOH: hydrophilic	< 10°	0.283 ± .054

The beads were pre-adsorbed with FXIIa or BSA from pure solutions and the amount of bound protein was calculated from depletion measures (data not shown).

Results of in vitro FXIIa-titration in dPPP are displayed in Fig 1. The CT corresponding to varying amounts of exogenous FXIIa introduced in different forms- bound to three different types of surfaces and as soluble FXIIa was recorded. The data was fit to a previously-developed mathematical model used to quantify the response of FXIIa-titration in plasma [2] in terms of three parameters as summarized in Table 2. As a negative control, BSA pre-adsorbed beads (with protein coverage equivalent to the FXIIa-beads) were titrated in dPPP. They did not elicit a significant coagulation response over the background response from the PS vial (data not shown).

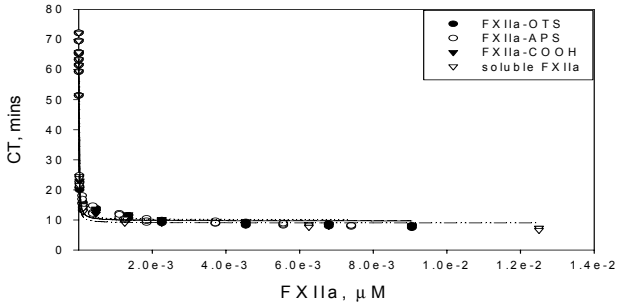

Figure 1: FXIIa-titration to dPPP

Table 2: Parameters from fit to FXIIa-titration to dPPP

FXIIa	a	b	C
Soluble	9.0 ± 1.2	(2.9 ± 0.7) x 10^{-4}	(4.7 ± 1.1) x 10^{-6}
OTS	9.8 ± 0.8	(4.0 ± 0.9) x 10^{-4}	(6.4 ± 1.5) x 10^{-6}
APS	10.1 ± 0.8	(4.0 ± 0.8) x 10^{-4}	(6.3 ± 1.5) x 10^{-6}
COOH	9.6 ± 0.8	(4.6 ± 0.9) x 10^{-4}	(7.0 ± 1.5) x 10^{-6}

Discussion: The three parameters in Table 3 describing the response to FXIIa –titration indicate that FXIIa introduced in all the different forms- bound to different surfaces and as soluble enzyme- are similar, indicating that FXIIa elicits a similar coagulation response. As a result of the experimental design, FXIIa was bound to the surface, so the molecules could not undergo the repeated adsorption-desorption steps believed necessary for propagation of coagulation. Neither the presence nor the properties of the underlying solid substrate had an effect on the procoagulation efficiency of the FXIIa, in contrast to the need for specific interactions with negatively charged surface suggested previously.

Conclusions: Comparison of surface-bound FXIIa procoagulant efficiency suggests that the surface properties do not affect procoagulant efficiency of the enzyme. Moreover, in contrast to other theories, repeated adsorption-desorption of the enzyme is not essential for coagulation, suggesting that the surface is necessary for activation, but not propagation of the cascade.

References:

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