Factor XII Contact Activation Products Show Self-Inhibition

Li-Chong Xu[a], Yuanjing Xu[b], Erwin A. Vogler[c], Christopher A. Siedlecki[a,c]
Department of [a]Surgery and [c]Bioengineering, The Pennsylvania State University, College of Medicine, Hershey, PA, 17033.
[b]Hershey High School, Hershey, PA 17033

Statement of Purpose: Blood coagulation resulting from contact activation due to blood – material interactions remains a challenge in the use of blood-contacting devices. The initiating step of the intrinsic pathway of plasma coagulation cascade is widely accepted to be surface-induced contact activation of the blood zymogen FXII into an active-enzyme form (FXIIa). This activation generates a suite of protein fragments showing procoagulant and amidolytic activities, and also suppression of FXIIa activity[1]. This work seeks to further investigate these inhibition properties of the FXII contact activation products towards identifying protein fragments generated FXII contact activation. Results show products of activation inhibit the procoagulant activity of FXIIa, and inhibition is moderated by prekallikrein (PK) and activator surface energy.

Methods: Glass beads were rigorously cleaned in aqua regia and piranha solutions, respectively, and rinsed with copious amount of DI water. Clean glass beads were either used as model hydrophilic surfaces, or were treated with octadecyltrichlorosilane (OTS) to prepare hydrophobic surfaces[2]. FXII at 30 μg/mL was activated in a polystyrene tube containing 100 mg of either clean glass or OTS-coated glass beads in PBS buffer, by mixing on a hematology mixer. An *in vitro* coagulation assay was used to measure the procoagulant activity of the products of FXII contact activation or interaction with exogenous FXIIa as measured by coagulation time (CT)[2]. To assay the inhibitory property of FXII activation products, the products and a mixture of products and beads were incubated with exogenous FXIIa for 10 min, then the procoagulant activity was assessed by measurement of CT. To investigate the role of prekallikrein in contact activation, human PK (20 μg/ml) was incubated with FXII (30 μg/ml) and 100 mg of appropriate bead type, and the inhibition of procoagulant activity of the related products was measured using the same method.

Results / Discussion:

Time course of inhibition of FXIIa activity by FXII contact activation with surfaces. Exogenous FXIIa was added during FXII contact activation with hydrophilic and hydrophobic glass beads. During period I (0-30 min, no extra FXIIa addition), coagulation time of plasma dropped quickly from 40.5 ±1.8 min for background, to 29.1 ±0.4 min and 30.6 ±0.5 min for hydrophilic and hydrophobic surfaces, respectively, indicating FXIIa generation. FXIIa was added during Period II (30-70 min, 1 μg/ml) and Period III (70-110 min, 2 μg/ml). A small drop in CT compared to Period I was seen after addition of exogenous FXIIa, however, the measured CT values were much higher than those of CT corrsponding to pure FXIIa activity in absence of surface activated products (Fig. 1). Furthermore, there is no significant difference in CT during periods II and III ($p>0.05$) although additional FXIIa (2 μg/ml) was added. Results suggest that the procoagulant activity of the exogenous FXIIa was inhibited by the FXII contact activation products.

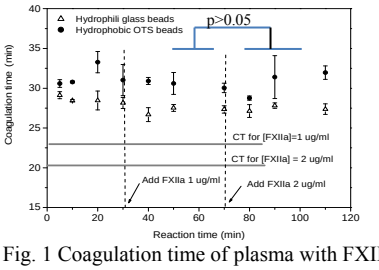

Fig. 1 Coagulation time of plasma with FXII activations and dosage of FXIIa at time 30 and 70 min.

Inhibition of procoagulant activity of FXIIa by supernatant products of FXII activation. The activation products following FXII contact activation were reacted with exogenous FXIIa for 10 min, and the activity of this exogenous FXIIa was significantly inhibited by the products. The inhibtion exhibited some non-stoichiometry, with higher doses of exogenous FXIIa showing greater greater reductions in activity. The inhibition of FXII contact activation products were quite similar for the hydrophobic and hydrophilic activators (Fig. 2). The presence of PK during FXII activation was found to increase the amount of FXIIa activated on hydrophilic and hydrophobic surfaces. The apparent inhibitors in FXII activation on hydrophilic surfaces appeared suppressed, but this could also be due to increased procoagulant FXIIa in this situaiton. The inhibitors produced by hydrophobic surface were not changed.

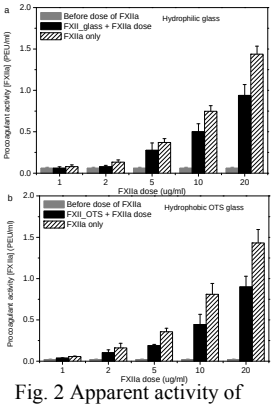

Fig. 2 Apparent activity of supernatant products of FXII contact activation dosed with FXIIa (a) hydrophilic and (b)hydrophobic surfaces.

Inhibition of FXIIa activity by mixtures of glass beads and supernatants of FXII activation. The mixture of activation products and activating beads was incubated with FXIIa (5 μg/ml) for 10 min and the procoagulant activity of the products was compared to FXIIa incubation in the absence of particles. Results showed that the mixtures of beads and products led to suppressed activity of FXIIa more than did only the activation products. Furthermore, regardless of surface energy and the presence of PK, the inhibition produced by mixture of beads and activated FXIIa products reached the similar value of ~85% reduction.

Conclusion:

Contact activation of FXII in buffer solution produces a suite of protein fragments including enzymes that exhibit procoagulant and amidolytic activity, and also molecules that inhibit the activity of FXIIa. The yields of inhibitors and inhibition rates are affected by the presence of PK and the activator surface energy. The findings in this work provide more insight into the mechanisms of FXII autoactivation that will be useful in the surface engineering of materials with improved hemocompatibility.

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References:

1. Golas et al., Biomaterials, 2011, 32, 9747.
2. 2. Chattejee et al., Biomaterials, 2006, 27, 5643.