Contributions of Prekallikrein and High Molecular Weight Kininogen to the Intrinsic Pathway of Coagulation

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Statement of Purpose: Occlusive thrombi can result from the undesired activation of the intrinsic coagulation cascade. This pathway activates when Factor XII (FXII) contacts a biomaterial surface, resulting in a group of protein fragments collectively known as FXIIa[1-2]. While autoactivation generates a small amount of FXIIa, much more is made by reciprocal activation, during which Kallikrein (Kal) that is generated by FXIIa activation of prekallikrein (PK), activates large amounts of FXII[3]. High-molecular-weight-kininogen (HMWK or HK) is thought to bring all coagulation factors into reactive proximity[1], though its precise role is currently uncertain. The objective of this study was to investigate the influence of PK and HMWK on intrinsic coagulation pathway.

Methods: Glass beads were rigorously cleaned in aquaregia and piranha solutions, and rinsed with large amount of DI water. After drying, one portion of clean glass beads was used as a hydrophilic Type II activator, while the remaining were modified with octadecyltrichlorosilane (OTS) (Type I activator) or 3-aminopropyltrichlorosilane (APTES) (Type 0)[2]. 500 µL of 30 µg/ml FXII in PBS was brought into contact with activators with or without prekallikrein and/or HMWK for 1 hr on a rotating hematology mixer. Yield of Kal was evaluated with time by commercial chromogenic assays. FXII autoactivation was assessed by plasma coagulation time assay for procoagulant yields, and chromogenic assays of cleaving Pefa-5963 for amidolytic yields.

Results / Discussion:

Kinectis of Kal generation (Figure 1). The rate of Kal generation is surface energy dependent initially (t< 10 min) in the order : clean glass > OTS > APTES. This trend is consistent with the yield of FXIIa by activators of different water wettability. Plateau levels of Kal formation were reached after 10 min incubation at clean glass, 30 min at OTS and 40 min at APTES. The presence of HK appeared to acclerate the conversion from PK to Kal, and reduce the differences in maximum Kal yield from three activators. These results confirm a function of HK as a cofactor in Kal generation.

Effects of PK and HK on FXII autoactivation (Figure 2). Adding PK increases the yield of both amidolytic and procoagulant fragments likely via reciprocal activation. Spefically, procoagulants at clean glass were amplified by 6.5 times in the presence of PK, which in turn strengthens the efficiency of FXII autoactivation by hydrophilic glass more than OTS and APTES. However, PK appeared to attenuate these differences in amidolytic yield produced by contact activation with activators of varying surface energy. Additon of HK has little effect of FXII autoactivation.

In conclusion, surfae-energy dependence of Kal generation initially is correlated with the amount of FXIIa produced by FXII autoactivation. Maximum yields of Kal by different activators were not significantly different from each other. Kal-FXII reciprocal activation shows different effects on procoagulang and amidolytic yields. HK appears to speed Kal generation, but has little effect on FXII autoactivation.

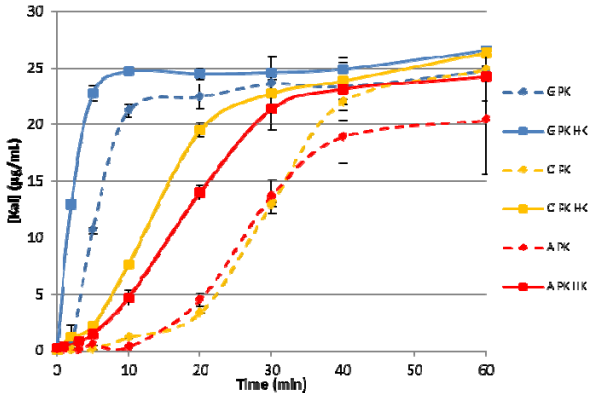

Figure 1. Kinetics of Kal generation with (----) or without (—) HK

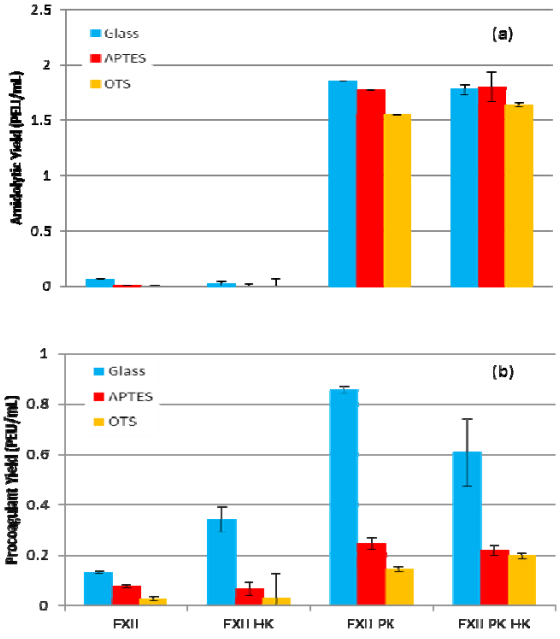

Figure 2. Yield of amidolytic (a) and procoagulant (b) fragments with or without PK or HK.

Reference:

1. Vogler and Siedlecki, Biomaterials, 2009, 30, 1857
2. Golas et al. , Biomaterials, 2011, 32, 9747
3. Chattejee et al., Biomaterials, 2009, 30, 4915