Solution-phase interaction of FXII and Prekallikrein in material-induced coagulation

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Statement of Purpose: A common problem with the use of blood-contacting medical devices is the activation of blood factors, which affects not only coagulation and thrombosis, but also the complement system, fibrinolytic pathways, and inflammatory responses in the body. Towards developing hemocompatible materials there is a need to understand the role of the material surface in contact activation events. Previous studies suggest contact activation of blood coagulation is initiated by binding of factor XII (FXII) to a negatively charged surface, which then facilitates subsequent reciprocal activation of Prekallikrein (PK) and FXII [1]. However, a recent study showed that FXII activation is not specific to hydrophilic surfaces [2]. In this study, Kallikrein (Kal) generation was measured after exposure to test surfaces and an alternate model of the role of surfaces in contact activation is proposed.

Methods: The two model material surface were used in this work: hydrophilic glow-discharge-cleaned 5 mL glass vial and octadecyltrichlorosilane (OTS)-modified glass vials [2]. An additional condition referred to as OTS-BSA was prepared by incubation of OTS vials overnight with 100 mg/mL BSA and PBS rinsing to "block" the surface. Time course generation of Kal from solutions of 0.2 μg/mL FXII or FXIIa with 20 μg/mL PK in PBS buffer contained in either glass, OTS, and OTS-BSA vials was measured using the chromogenic substrate Pefachrome-PK (Centerchem Inc, Norwalk, CT). 15 μL aliquots of the protein solution from the vials were removed at the indicated time points, incubated for 10 min at 37°C in a 1.5 mL disposable plastic cuvette containing 1066 μL of Tris-Immidazole buffer (pH 7.8), 150 μL of 0.5 mM Pefachrome-PK and 15 μL of 14.5 μg/mL Corn Trypsin Inhibitor. The reaction was stopped with 300 μL of 10% glacial acetic acid and absorbance measured at 405 nm.

Results/Discussion: Fig. 1 shows the time course of Kal generation from the PK-FXIIa system appears similar for glass and OTS-BSA, but greatly reduced for OTS, indicating that the strongly-adsorbent hydrophobic OTS surface inhibits activation of PK by FXIIa. Further, the addition of 400 mm^2 of OTS beads (0.5 mm diameter) to the glass vial (designated Glass+OTS) can be used to "inhibit" the solution-phase PK activation in glass vials presumably by adsorbing active enzyme and decreasing the solution phase enzyme available.

Kal generation from PK-FXII system (Fig.2) indicates that large amounts of Kal are generated only in glass vials but not in OTS or OTS-BSA. In conjunction with the previous publication [2], this result suggests that an initial contact with a surface is essential for the production of FXIIa, which in turn cleaves PK to Kal in solution. While the first step occurs in both glass and OTS as reported previously [2], this step is possibly prevented by the adsorbed BSA in OTS-BSA. However, on the hydrophobic OTS surface, the FXIIa generated is not efficiently released back into solution for subsequent PK cleavage. However, by providing solution FXIIa to PK in the OTS-BSA vial, solution phase Kal activation can occur, even in the hydrophobic vial, as shown in Fig. 1.

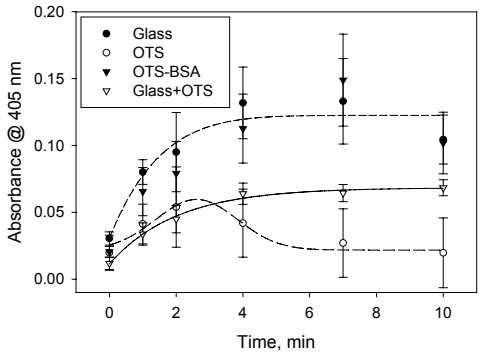

Fig. 1: Absorbance at 405 nm from Kal generation in 20 μg/mL PK + 0.2 μg/mL FXIIa.. Lines are guide-lines drawn for the eyes

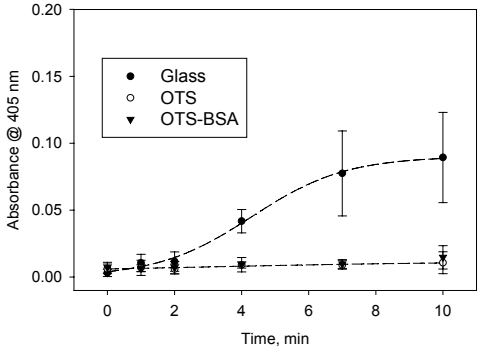

Fig. 2: Absorbance at 405 nm from Kal generation in 20 μg/mL PK + 0.2 μg/mL FXII. Lines are guide-lines drawn for the eyes

Conclusions: Traditional biochemistry of contact activation suggests that hydrophilic surfaces are activators because they "specifically" bind FXII and PK, and facilitate reciprocal-activation by protein assembly on the surface, while hydrophobic surfaces lacking the negative charge are inefficient activators. In contrast, results here indicate that reciprocal-activation is a solution phase interaction. While all surfaces are potential activators [2], strongly-adsorbent hydrophobic surfaces leads to protein adsorption and depletion of proteins in solution, thereby inhibiting solution-phase reciprocal-activation. Weakly-interacting hydrophilic surfaces are apparent activators not because they are specific but because they do not inhibit solution-phase molecular interactions.

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