Effect of Competitive Protein Adsorption on Functional Activity of Adsorbed Fibrinogen Measured by AFM

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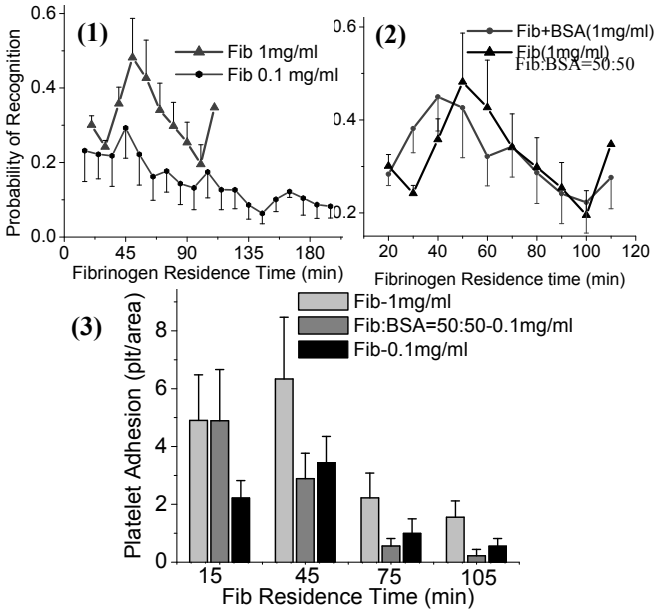
Statement of Purpose: Platelet adhesion to a biomaterial surface requires fibrinogen undergoes conformational changes that expose the platelet binding epitopes, particularly the γ-chain dodecapeptide (γ400-411). Previous studies in our lab used atomic force microscopy (AFM) to study the functional changes in fibrinogen on mica substrates [1]. In this study, a monoclonal antibody (mAb) that recognizes fibrinogen γ392-411, including the γ-chain dodecapeptide, was coupled to the end of an AFM probe. Interactions between the modified probe and surface were used to study the effects of concentration and coadsorption with bovine serum albumin (BSA) on the functional changes in adsorbed fibrinogen.

Methods: Triangular silicon nitride cantilevers with integral probes (k~0.06N/m) were modified with mAbs recognizing γ-392-411, through a standard protocol [1]. Bovine serum albumin (BSA, Sigma Chemicals Co, St. Louis, MO) and human fibrinogen (Calbiochem, La Jolla, CA) were incubated on muscovite mica (Ted Pella Inc., CA) in varying concentrations (0.1mg/ml & 1mg/ml) and as mixtures (50:50 by weight) for residence-times of 5-180 minutes in a fluid cell and then exchanged for fresh buffer at a steady flow of PBS at 0.3ml/min.

All data was collected using a Nanoscope III Multimode AFM (Digital Instruments, CA) under buffer conditions with varying scan sizes ($500nm^2$-$5000nm^2$) and scan rates (0.5Hz – 1 Hz). Images were collected in 32x32 pixel format with 256 data points per force curve. Interactions between fibrinogen and the antibody were then characterized as either specific or nonspecific using a 95% confidence interval calculated from control data generated for each probe. Adhesion data from multiple runs were compiled and the probability of the antibody encountering the antigen at any post-adsorption time (± 30 sec) calculated.

Whole blood from a healthy human donor (EDTA anticoagulant) was used to prepare platelet rich plasma. Washed platelets were prepared by a series of centrifugation and exchange procedures. Mica samples were subjected to 5 min fibrinogen incubation time and varying residence times (5-180min) and then incubated exposed to platelet suspensions ($250x10^3/\mu l$). Platelets were labeled with fluorescent antibodies and the platelets were counted using optical microscopy.

Results / Discussion: Fig 1 illustrates the time-dependence in the probability of antigen recognition for a the region that includes the platelet binding dodecapeptide. For 0.1 mg/ml concentration, the maximum likelihood of adhesion occurs ~ 45 min residence time. At longer adsorption times, the probability of adhesion decreases dramatically. With increased concentration (1mg/ml) the probability increases with the recognition peak occurring at ~50 min fibrinogen residence time. This increase in the probability is presumably due to increases in the number of available binding sites. Coadsorption of BSA with fibrinogen leads to a shift in the recognition peak as illustustrated in Fig 2. Results from Fig 3 demonstrate that platelet adhesion peaks at 45min fibrinogen and decreases with increasing residence time for both concentrations of fibrinogen. These results correlate very well with the AFM data. However, when coadsorbed with BSA, the peak shifts dramatically to the earlier time points suggesting that the dodecapeptide sequence are most active or exposed for binding ~15min and decrease with increasing residence time. Although we see a shift in the maximum activity of fibrinogen in fig 2, that shift is very small with peak occurring at ~40min as compared to the macroscale platelet adhesion results where the shift in the peak is from ~45 min to ~15min. Several control experiments have been performed confirming that this is a time-dependent surface process rather than an artifact of antibody degradation after extended periods of measure. We continue to perform experiments using various concentrations and ratios on both model substrates and clinically relevant polymer substrates.



Summary: A monoclonal antibody recognizing a region that includes the platelet binding epitope of the γ chain dodecapeptide (γ400-411) was used to measure the effects varying concentration and competitive protein adsorption on fibrinogen functional activity following adsorption. This data can provide us a better understanding of the biological activity of fibrinogen at a molecular scale.

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References: (1) Soman, Langmuir, 24, 2008.