Adsorption Does Not Enhance Exposure of the Fibrinogen Gamma Chain Dodecapeptide

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Statement of Purpose: In solution, platelets aggregate upon the binding of their αIIbβ3 receptors to the fibrinogen gamma chain dodecapeptide (GCDP) (γ400-411).[1] Conformational changes in adsorbed fibrinogen may enhance the exposure of platelet adhesive sites that are inaccessible in solution, allowing adhesion of unactivated platelets to surfaces.[2] However, it is not clear if the GCDP is involved in the adhesion of unactivated platelets to adsorbed fibrinogen. In principle, because unactivated platelets cannot bind to the GCDP in solution, the GCDP must become more exposed upon adsorption if it is involved in the adhesion of unactivated platelets to substrates. To test this hypothesis, we developed mass spectrometric methods to quantify chemical modifications of specific residues in fibrinogen following adsorption to biomaterial surfaces.

Methods: The quantitative method used an internal standard consisting of isotope-labeled fibrinogen secreted by human HepG2 cells grown in culture in medium containing ^{13}C-leucine. The isotope-labeled fibrinogen was purified by immunoaffinity chromatography. On the immunoaffinity resin, lysine residues within the internal standard fibrinogen were reacted with NHS-biotin for 5 min. For the experimental samples, normal human fibrinogen was adsorbed to polyethylene terephthalate (PET) particles. The adsorbed fibrinogen was reacted with NHS-biotin for 5 min and eluted from the surface. Constant amounts of internal standard were added to the adsorbed fibrinogen samples and analyzed by ion trap mass spectrometry. The ion intensity of the elution peak of the GCDP was integrated and compared between the sample and the internal standard to determine the extent of biotinylation of the GCDP in the various samples.

Results: We previously found by qualitative mass spectrometry that sites adjacent to the GCDP became more exposed following adsorption to PET, but results with the GCDP were inconclusive.[3] Previous antibody binding studies yielded similar results.[4] We developed new mass spectrometric methods to quantitatively measure the extent of biotinylation of the GCDP, which we used as a measure of the amount of exposure of the lysine in the GCDP. The use of ^{13}C-isotope internal standard greatly enhances the reliability of quantitation by mass spectrometry, as we have previously shown.[5]

Approximately 80% of the GCDP peptides became biotinylated when fibrinogen was reacted with NHS-biotin in solution. However, the same was also true for fibrinogen bound to the immunoprecipitation resin, or adsorbed onto the PET surfaces (Fig. 1). For the adsorbed fibrinogen, the solution concentration potentially could profoundly effect the conformational state of the protein. The spread area of fibrinogen increases dramatically as the solution concentration decreases.[3] This is a result of the balance between the kinetics of adsorption and the kinetics of 'unfolding' on a surface. At lower solution concentrations, more time is required to reach a dense packing of protein. Adsorbed proteins thus have more space and time to spread to a larger area. However, examining a range of solution concentrations, we found no evidence that adsorption of fibrinogen to PET results in greater accessibility of the lysine in the GCDP to NHS-biotin.

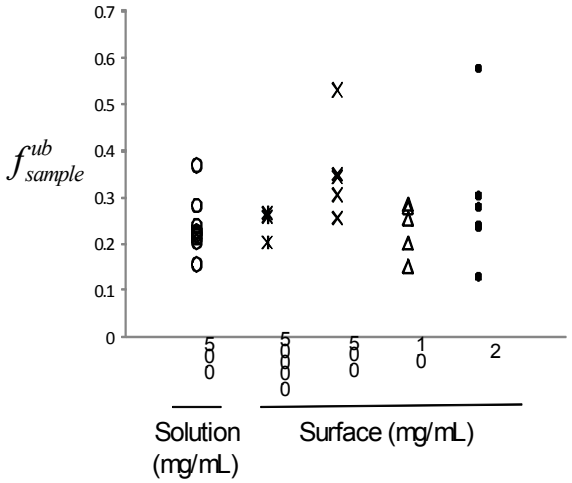

Figure 1: Measurement of the fraction of the GCDP that was unbiotinylated following incubation with NHS-biotin for 5 min. Fibrinogen was either in solution at 0.05 mg/mL or adsorbed to the surface of PET particles from solutions of different concentrations. No evidence was found for increased biotinylation in adsorbed fibrinogen.

These results are generally consistent with previous antibody binding studies and suggest that other regions of fibrinogen may be crucial in promoting platelet adhesion to materials. One limitation of the current study is the small size of the probe compared to the size of integrin receptors or antibodies. Steric effects may limit access to the GCDP. Further study of conformational changes in the rest of the fibrinogen may provide insight.

Conclusions: The mechanism of platelet adhesion to adsorbed fibrinogen is still poorly described at the molecular level, but these results suggest that the GCDP should only promote the adhesion of activated platelets to surfaces.

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