Mass Spectrometric Mapping to Investigate Protein Conformations at Solid/Liquid Interfaces

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Statement of Purpose: Conformational changes occur in proteins after adsorption to biomaterial surfaces. These time-dependent changes affect protein surface coverage and expose hidden binding sites that are responsible for eliciting biological responses.[1,2] Fibrinogen interactions with biomaterials direct cellular and thrombotic responses on the materials. A better understanding of fibrinogen conformational changes at the molecular level may provide information useful in the development of devices with enhanced biocompatibility. We have developed a new method involving chemical labeling of adsorbed proteins and mass spectrometric mapping to characterize the adsorption of fibrinogen onto poly(ethylene terephthalate) (PET). We also used optical waveguide lightmode spectroscopy (OWLS) to detect global changes in protein conformation as a function of the solution concentration of fibrinogen.

Methods: *OWLS:* Spin coating was used to apply a 50 to 75 nm thick layer of PET onto OWLS waveguide chips. Thickness of the layers was verified by ellipsometry and and composition was verified by X-ray photoelectron spectroscopy. The adsorption of fibrinogen from 0.1, 0.5 and 1 mg/ml solutions in PBS, pH 7.4 was monitored using a MicroVacuum OWLS 110. The thickness and density of the adsorbed layers were calculated and the change in surface density over time (dM/dT) was determined for each time point.

Mass Spectrometric Mapping: PET particles (1- 200 μm diameter, surface area of 21.9 cm^2/mg) were incubated with 0.1, 0.5 or 1 mg/ml solutions of fibrinogen in PBS, pH 7.4. Adsorbed proteins were labeled at lysine residues with NHS-LC biotin (Pierce Chemicals). Proteins were eluted from the PET with 4% sodium dodecyl sulfate in PBS buffer. The labeled fibrinogen was purified by SDS-PAGE, digested with trypsin and analyzed by MALDI-TOF mass spectrometry. The resulting spectra were analyzed with in-house Perl scripts that identified masses corresponding to labeled fibrinogen peptides.

Results/Discussion: A concentration-dependent conformational change of fibrinogen was evident on PET by OWLS. Our results showed that the mass density of fibrinogen on PET after incubation with 1 mg/ml fibrinogen was 275.48 ± 4.34 ng/cm^2, while a 0.1 mg/ml solution resulted in 119.63 ±. 22.82 ng/cm^2 of fibrinogen. MALDI-TOF analysis revealed locations of lysine labeling, and most of these locations were verified with ion trap mass spectrometry (confirmed sites are labeled with crosses in Fig. 1). Chemical labeling of fibrinogen γ chain was quite similar between solution and surface, but revealed a statistically significant change in lysine γ381 labeling when fibrinogen was adsorbed from 0.1 mg/mL fibrinogen (Fig. 1). This, along with the identification of γ406 only in adsorbed fibrinogen suggests a higher exposure of C-terminal γ chain residues when fibrinogen is able to more fully spread on surfaces. This result is consistent with the role of the γ chain C-terminal dodecapeptide in promoting platelet adhesion to artificial materials.[2]

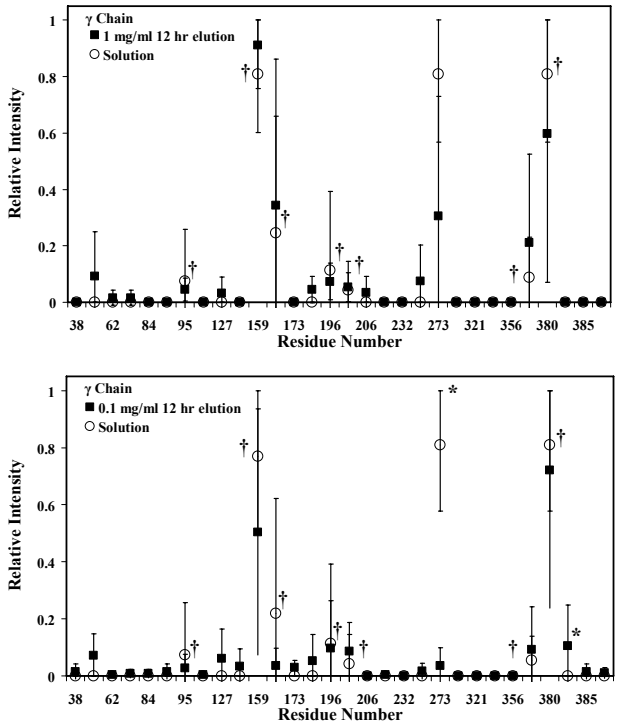

Fig. 1: Relative MALDI spectral peak intensities of NHS-LC-Biotin labeled lysine residues within fibrinogen γ chains after a 1 mg/ml (top) and 0.1 mg/ml (bottom) incubation with PET particles. Wilcoxon rank sum statistics were used to compare locations labeled after adsorption with locations labeled while in solution. Statistically significant changes are marked with an (*). Solution labeled residues observed in both MALDI and ion trap MS are marked with an (†).

Conclusions: We were able to calculate the average surface area of individual fibrinogen molecules upon adsorption and our results correlate well with atomic force microscopy measurements of fibrinogen in a spread state on hydrophobic surfaces[3]. Chemical labeling suggested the possible exposure of a biologically-relevant domain in fibrinogen that is hidden in solution. Additional investigations using quantitative mass spectrometric methods may further define conformational changes in adsorbed fibrinogen.

References:

[1] Ramsden JJ. Phys Rev Lett. 1993; 71(2):295-298.
[2] Farrell, D.H. et al., J. Biol Chem. 1994, 269, 226.
[3] Sit, P.S. et al., Thromb Hemost 1999; 82: 1053-60