Delineating the Specific Interactions Mediating Platelet Adhesion to Adsorbed Plasma Proteins

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Statement of Purpose: Recent studies by our group have shown that platelet adhesion to both adsorbed fibrinogen (Fg)[1] and albumin (Alb)[2] correlates very strongly with the degree of adsorption-induce protein unfolding but not with the amount of protein adsorbed. The results illustrate the critical role played by the adsorbed conformation of proteins in mediating platelet adhesion. These results are particularly intriguing, as Alb is conventionally thought to be unable to bind platelets and has even been used as a hemocompatible coating on biomaterial surfaces.[3] The objective of this research was to probe the mechanisms mediating these types of interactions for both Fg and Alb.

Methods: Self-assembled monolayers (SAMs) of CH_3- and OH-terminated alkanethiols (Sigma-Aldrich) were formed on gold-coated cover glasses. Human Fg (FIB3, Enzyme Research Labs) and Alb (Sigma-Aldrich) were preadsorbed at 0.1 mg/mL solution concentrations on these SAM surfaces. Various blocking strategies were used on the adsorbed protein layer and the washed platelets prior to platelet adhesion. Treatments included RGDS/RGES, Arginine (Arg)-modification of adsorbed Alb, and Aggrastat ($\alpha_{IIb}\beta_3$ receptor-inhibitor drug). Platelet adhesion was quantified using a lactate dehydrogenase (LDH) assay, while their adherent morphology was visualized using a S4800 field-emission scanning electron microscope.

Results: The role of non-specific interactions in platelet adhesion was ruled out by the fact that anti-Alb polyclonal antibodies (pAb-Alb) nearly completely inhibited platelet adhesion to adsorbed Alb, while anti-Fg polyclonal antibodies (pAb-Fg) had no significant effect. Similarly, pAb-Fg inhibited platelet adhesion to adsorbed Fg, while pAb-Alb was ineffective.

As seen in Fig.1, the RGDS peptide, which is known to bind to $\alpha_{IIb}\beta_3$ platelet receptors, inhibited platelet-protein interactions on both Fg and Alb (RGES had no significant effect), suggesting that this receptor is involved in platelet adhesion to both of these proteins. For a given SAM, the extent of RGDS-based inhibition was nearly identical (~60% on CH_3, ~45% on OH) for Alb and Fg. The fact that RGDS only partially blocked platelet adhesion suggests that non-RGD specific platelet receptors may also play a role in platelet adhesion to both adsorbed Fg and Alb. Fig. 2 shows SEMs of the platelets on each type of adsorbed protein with and without RGDS. While platelets without RGDS treatment were activated on both proteins, activation was much stronger on adsorbed Fg. Platelet morphology following RGDS-treatment, which shows adhesion without activation (Fig. 2.B,D), is much more similar on both proteins, providing evidence that the platelet adhesion mechanisms are similar in each of these cases. This adhesion process may be mediated by $\alpha_2\beta_1$ integrins, which have been reported to be activated by RGDS treatment,[4] leading to platelet adhesion without overall activation. Neutralization of Arg residues by chemical modification of adsorbed Alb (Fg studies underway) also caused a significant decrease in platelet adhesion levels, thus indicating the involvement of Arg amino acids in the binding motif in Alb that is recognized by the platelet integrins. Aggrastat pretreatment of platelets led to an even greater decrease in platelet adhesion than RGDS-treated platelets or Arg-modified Alb, further implicating $\alpha_{IIb}\beta_3$ as the primary receptor involved in these platelet adhesion processes.

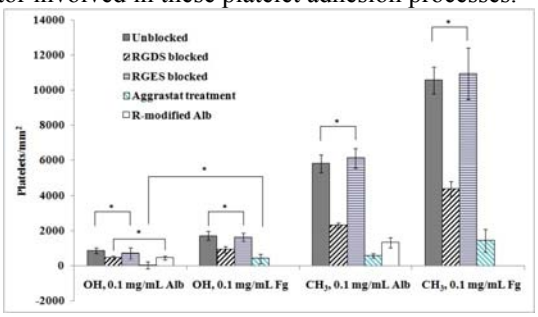

Fig.1. Platelet adhesion to Alb and Fg preadsorbed on SAM surfaces from 0.1 mg/mL solutions under various blocking conditions. (n = 6, mean ± 95% CI). * denotes no statistically significant difference, $p > 0.05$.

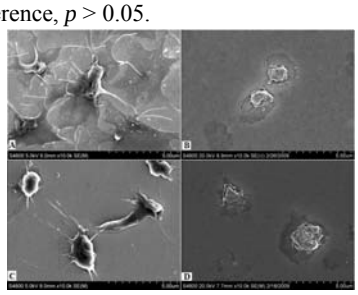

Fig.2. Morphology of adherent platelets on CH_3 SAMs preadsorbed with Fg (top row) and Alb (bottom row), without (A,C) and with (B,D) RGDS-pretreatment.

Conclusions: The combined results of platelet adhesion inhibition caused by platelet treatment with RGDS (but not RGES) and Aggrastat indicates that platelet adhesion to both Fg and Alb under these conditions occurs by receptor mediated processes, with the likely involvement of the $\alpha_{IIb}\beta_3$ platelet receptor. The large reduction in platelet adhesion by modification of the Arg residues in Alb indicates their involvement in these receptor-mediated processes as well. The differences in morphology of RGDS-blocked and unblocked platelets suggests that platelet adhesion to Fg and Alb may be mediated by two distinct receptor sets; an RGD-specific set mediating both adhesion and activation and a non-RGD-specific set that induces adhesion with minimal activation. Overall, there is much to learn about the molecular mechanisms underlying platelet-protein interactions. Future studies are planned to further delineate the platelet receptors and the protein motifs that mediate platelet interactions with adsorbed Fg and Alb.

References: (1) Sivaraman B. *Biomaterials*, doi:10.1016/j.biomaterials.2009.10.008. (2) Sivaraman B. *Biomaterials*, doi:10.1016/j.biomaterials.2009.10.017. (3) Marois Y. *Biomaterials* 1996, 17:3-14. (4) Van De Walle GR. *Blood* 2007, 109: 595-602.