Interactions of the Platelet Integrin Receptor GPIIbIIIa with Surface-Adsorbed Fibrinogen

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Introduction: Binding of the platelet receptor protein GPIIbIIIa (integrin $\alpha_{IIb}\beta_3$) to biomaterial surface-adsorbed fibrinogen is a critical event in blood-material interactions and surface-induced thrombogenesis. In this study, the interactions between purified platelet membrane receptor GPIIbIIIa with fibrinogen adsorbed to model hydrophilic (mica) and hydrophobic (highly ordered pyrolytic graphite, HOPG) surfaces were characterized by measuring ligand-receptor debonding forces using atomic force microscopy (AFM).

Methods: Purified integrins (Enzyme Research Labs) were directly immobilized on amine-modified integral tips using the long-thin Si_3N_4 triangular cantilevers (Digital Instrument) through a gluteraldehyde linker molecule. Fibrinogen (Calbiochem, CA) at 10 μg/mL was adsorbed onto substrates for 1 h. Force data was acquired with a Nanoscope IIIa Multimode AFM (Digital Instruments, CA) in adhesion mode and under buffer using integrin-modified probes against surface-adsorbed fibrinogen. Loading rates were varied between 10-60 nN/s. After acquiring force curves, integrins were blocked by an RGD-containing peptide (Sigma Chemicals), and force curves again acquired. In separate experiments, the force curves were acquired in a buffer containing 1 mM EDTA (a Ca^{2+} chelator that renders the integrin inactive). Control force data was taken against a BSA (Sigma Chemicals) layer adsorbed on mica to characterize non-specific protein-protein interactions. Force data was extracted and analyzed off-line with tools developed in MatLab™ to estimate the debonding strength of single integrin-fibrinogen pairs.

Results / Discussion: Rupture events between integrins on a tip and adsorbed fibrinogen were observed over a large range of distance from the surface. The distributions of rupture length showed that on HOPG the rupture events occurred in the range of 20-200 nm from the surface while the range of rupture length on mica was 20-400 nm. These long ranges when compared to the dimensions of fibrinogen and integrins suggest that fibrinogen was likely undergoing mechanical denaturation during the force cycles. The difference in the range of rupture lengths on mica and HOPG indicates that at least a part of fibrinogen population is undergoing stretching to a greater extent on mica than on HOPG.

To determine the strength of a single ligand-receptor bond, statistical distributions of the force corresponding to the rupture events were plotted for each loading rate. The distributions of rupture forces showed several peaks, however, clear periodicity corresponding to the debonding strength of a single pair was not apparent. This suggests that the rupture events may be also due to rupture of proteins interacting through non-specific interactions (as observed in a small fraction of force curves in control experiments) and mechanical denaturation of fibrinogen domains (as suggested by the long rupture lengths) in addition to the debonding of integrin-fibrinogen pairs. By making the assumption that the non-specific interactions does not contain any periodicity (verified in control experiments) and that the mechanical denaturation will not result in a periodic distribution of rupture force, it can be concluded that the rupture force values for integrin-modified probes and adsorbed fibrinogen contain a periodic distribution due to debonding of integrin-fibrinogen pairs with non-periodic 'noise' arising from the non-specific interactions and the mechanical denaturation of fibrinogen. The periodicities in the rupture force distributions that are attributed to the debonding force of a single fibrinogen-integrin pair were extracted by developing an algorithm that maximizes the number of peaks counted towards the estimation of the periodicity and are presented in Table 1.

Table 1: Debonding strength of a single integrin-fibrinogen pair at different loading rates.

Loading Rate (nN/s)	Debonging strength of integrin-modified probe against	
	fibrinogen adsorbed on mica (pN)	fibrinogen adsorbed on HOPG (pN)
10	57	63
30	63	52
60	70	68

Maximum error in the estimated debonding strengths = 20 pN

Comparison of the debonding strength at the same loading rates on mica and HOPG suggests that there is no difference in the debonding strength for integrins and fibrinogen adsorbed to these two surfaces. From this analysis, it can be concluded that the debonding strength of a single integrin-fibrinogen pair lies in the range of 50-80 pN for loading rates 10-60 nN/s on both surfaces, consistent with previous reports.[1]

Conclusions: The data in this study suggest that the interaction strength between platelet integrin receptors and adsorbed fibrinogen is independent of the surface properties of the material to which the protein is adsorbed. An important physiological implication of these results is that the surface properties of the material can then influence platelet adhesion only by modulating the expression of platelet-binding epitopes in the adsorbed fibrinogen, however, once the platelet-binding epitope is available, binding of the platelet membrane receptor will follow the same kinetics regardless of the surface properties.

References: 1. *Litvinov et al.* PNAS-USA 2002; 99, 7426-7431.