Photo courtesy Micelle A. Berny.

Thrombin Interactions with Clots Formed under Shear Flow: The Role of γ′-Fibrinogen

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To examine the mechanisms of thrombin binding to blood clots, clots were formed in vitro by co-perfusion of reconstituted blood (platelets, red blood cells, and fibrinogen-deficient plasma supplemented with γA- or γ′-fibrinogen) with Ca^{2+}/Mg^{2+}/tissue factor into capillary tubes. The role of γ′-fibrinogen, containing the high affinity thrombin-binding site, in thrombin-clot binding was investigated by forming clots in the absence or presence of γ′-fibrinogen, then post-labeling clots with fluorescently labeled (OG488) thrombin. As indicated in Figure 1, OG488-thrombin bound to clots formed with and without γ′-fibrinogen, suggesting that the low affinity thrombin-binding sites on fibrinogen may play an important role in thrombin-clot interactions.

Thrombin is generated from its precursor, prothrombin, by factor Xa (FXa) mediated cleavage. To further elucidate thrombin-clot interactions, we investigated the localization of proteins involved in the generation of thrombin. Clots were formed by co-perfusion of sodium citrate anticoagulated human blood with Ca2+/Mg2+/tissue factor into capillary tubes. Following formation, clots were labeled with OG488-labeled coagulation factors in combination with AF647-labeled annexin A5, which binds with high affinity to exposed phosphatidylserine (PS) on the surface of highly activated platelets. Importantly, PS has been shown to provide Ca2+-mediated binding sites for coagulation factors to assemble. In confirmation of this, our data show that the labeling of OG488-FXa on clots substantially overlapped with PS-exposing platelets (Figure 2). To evaluate this quantitatively, the Pearson's overlap coefficient, a value from -1 to +1 which ranks the pixel overlap of two fluorescent colors, was determined. Quantitatively, the overlap between annexin A5 and FXa was confirmed by a high Pearson's overlap coefficient, Rr, between labels of 0.56. In parallel, when post-labeling was performed with prothrombin, strong overlap between prothrombin and PS-exposing platelets was observed, resulting in a Pearson's correlation of 0.56 (Figure 2). In marked contrast, OG488-thrombin distributed over clots and fibrin fibers, with a low overlap (Rr = 0.10) with annexin A5 (Figure 2).

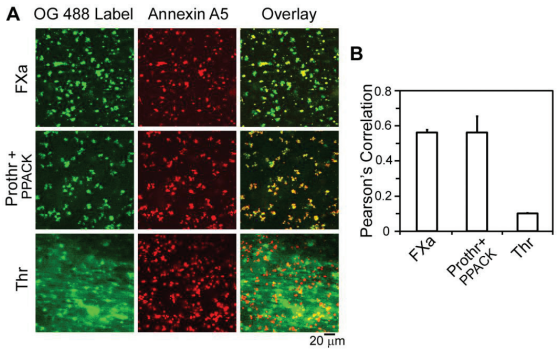

Figure 2. Localization of factor Xa (FXa) and pro(thrombin) on blood clots. Clots were formed by co-perfusion of blood with Ca2+/Mg2+/tissue factor and labeled with annexin A5 (red) or OG488-labeled coagulation factors (green). A, Fluorescent images of FXa, prothrombin (Prothr), thrombin (Thr), and annexin A5 binding. B, Pearson's correlation coefficient showing overlap between PS-exposing platelets and green labels. Mean±SEM.

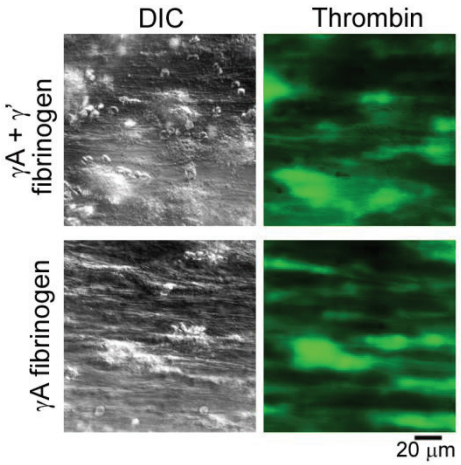

Figure 1. Clots formed in the absence or presence of γ'-fibrinogen support thrombin binding. Fibrinogen deficient plasma was supplemented with γA-and γ'-fibrinogen (top) or γA-fibrinogen (bottom) and added to washed platelets and red blood cells to make reconstituted blood. Reconstituted blood was co-perfused with Ca2+/Mg2+/tissue factor into capillary tubes. Formed clots were post-labeled with OG488-thrombin and imaged with differential interference contrast (DIC) and fluorescence microscopy.

Together, these results point to an initial binding of prothrombin to PS-exposing platelets, after which it redistributes to the platelet-fibrin thrombus once cleaved into thrombin.

In December 2010, I defended my graduate thesis, earning my Ph.D. in Biomedical Engineering. My studies on the mechanisms of thrombin-clot interactions, supported by the AAAS, were integral to my thesis.