Atomic Force Microscopy Study of Coagulation Factor XII Interaction with Factor XI Autoactivation on Hydrophilic and Hydrophobic Surfaces

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Statement of Purpose: Blood coagulation resulting from contact activation due to blood – material interactions remains a challenge in the use of blood-contacting devices. The initiating step of the intrinsic pathway of plasma coagulation cascade is widely accepted to be surface contact activation of the blood zymogen FXII (Hageman Factor) into an active-enzyme form. In plasma, increased contact efficiency is seen with with anionic, hydrophilic procoagulants. However, recent work demonstrates that hydrophobic and hydrophilic activators have nearly equal autoactivation properties in neat buffer solutions of FXII. These mechanisms remain unclear and motivate the further exploration of molecular interactions of FXII with other proteins such as coagulation factor FXI. In this study, AFM was employed to measure the interactions of activated FXII with FXI, with activated FXII produced by interactions with hydrophilic (mica) and hydrophobic (HOPG) surfaces.

Methods: Human coagulation Factor XI was immobilized on a mica surface. Briefly, the mica was incubated in 1% (v/v) APTES (aminopropyltriethoxysilane) for 1 hr, and then incubated in 10% glutaraldehyde for 1 hr after rinse. 50µl of a FXI solution was placed on mica surface for 1 hr. After rinsing in PBS, half the piece of mica surface was cleaved to provide a fresh mica surface for contact activation. Human coagulation factor XII was linked to Si_3N_4 AFM probes with same method. To measure the interaction forces between FXII and FXI upon contact activation, FXII-coated probe was first contacted with mica or HOPG surfaces 5 times, and then the probe was moved to FXI-coated surfaces for force measurements. Force curves (16×16) were collected by force volume image mode at speed of 1 Hz and ramp size of 1 µm within scan area of 5×5 μm^2. The non-activated FXII interactions were directly measured by probes interacting with FXI without prior surface contact. BSA was used as a control which was coated on mica surface with same method. AFM force data were extracted and analyzed by Matlab software. The probability of interactions between proteins was evaluated by rupture force and separation energy. Rupture force was calculated from the distance between the zero deflection value to the point of maximum deflection during probe separation from the surface and the separation energy is calculated from the summation of the total area in the retraction force curve.

Results / Discussion:
Interaction of FXII with proteins was measured on protein-coated surfaces through multiple FXII-coated AFM probes and replicated protein-samples, and a totals of thousands data points were collected for each case. Figure 1 illurtrates the cumulative percentages of rupture force and separation energy distributions to show the probablity of interactions between FXII and FXI or BSA. Surprisely, interactions between active and inactive FXII were occasionally measured on BSA-coated surfaces, although the number of these interactions were small. The distribution of rupture force and separation energies are similar for activated or non-activated FXII and BSA. Results show that ~70% of reaction points are with rupture force <0.1 nN, and ~75% of points are with separation energy<2.5 nN·nm (Fig. 1).

Compared to interactions between non-activated FXII and FXI, more interactions were measured between the FXI-coated surface with activated FXII probes that were first contacted with either mica or HOPG surfaces, suggesting that the activation of FXII to FXIIa by contact produced increased interactions with protein FXI. Because the range of rupture force and separation energy are large, the distribution of Ln(forces or energy) is illustrated in Fig. 2. A peak around Ln(force)=0 (i.e., rupture force = 1nN) appears in the distribution of interactions of FXII (activated on mica or HOPG) and FXI, while the peak for interactions of non-activated FXII and FXI is observed around Ln(force)= -2. The distrubtuion of Ln(separation energy) clearly shows two peaks, indicating the probablity of non-interactions and interactions on protein FXI could be distinguished at Ln(energy) of 0.5. Thus, the total probablity amounts of interaction are 55.6%, 51.7% and 44.9% for FXII contacted on mica, HOPG and non-contacted, respectively. Results suggest that the hydrophilic surface is slight more efficient for contact activation of FXII than on hydrophobic surface. Both are more efficent than non-contacted activation, but there are significant numbers of actived FXII from the coupling methods alone.

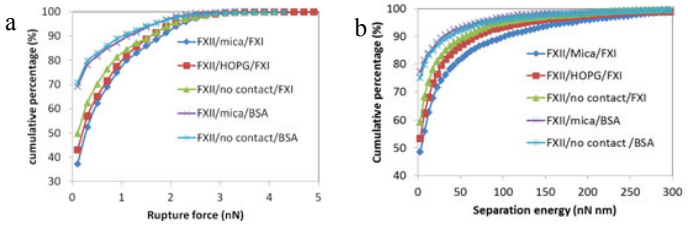

Fig. 1. Cumulative distribution of (a) rupture force and (b) separation energy of data points.

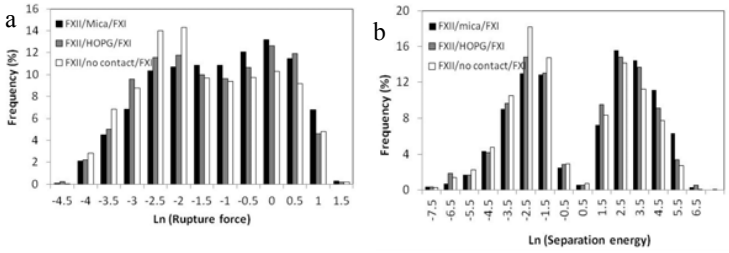

Fig. 2. Distribution of (a)rupture force and (b) separation energy of data points, treated with logarithm.

Reference:

1. Zhuo R. et al. Biomaterials, 2006, 27, 4325
2. Zhuo R. et al., Biomaterials, 2007, 28, 4355.