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Spreading of Fibrinogen at Model Surfaces Studied by AFM

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Fibrinogen is a crucial protein in surface-induced thrombus formation due to its importance in mediating platelet adhesion to biomaterials by serving as an adhesive ligand for the $\alpha_{IIb}\beta_{III}$ integrin receptor. The adsorption and function of fibrinogen have been widely studied by a variety of techniques, and have demonstrated both time- and material-dependent properties of the protein[1], and have contributed to our understanding of fibrinogen's interesting properties on biomaterial substrates. In this study, we have utilized atomic force microscopy (AFM) to examine the changes in the protein structure as a function of both time and material as a step towards an ultimate goal of understanding molecular level structure/function relationships. These studies demonstrate that fibrinogen denatures much more rapidly and to a greater degree on a hydrophobic model surface than on a hydrophilic surface, as might be expected. Furthermore, analysis of the individual D and E domains of the molecule indicate differences in the rate of structural change within the molecule itself, a phenomenon which may be associated with submolecular properties of the protein

Fibrinogen structure was examined on two substrates of varying surface wettability, highly ordered pyrolytic graphite (HOPG), a hydrophobic material with an advancing water contact angle over 100°, and muscovite mica, a hydrophilic material with an advancing water contact angle of <10°. These model materials were chosen as they not only possess vastly different surface properties, but also are smooth enough to permit nonambiguous imaging of individual proteins. Fibrinogen was added at concentrations ranging from 500 ng/ml to 2 μg/ml in 1 mM phosphate buffer for 15 minutes in order to yield submonolayer amounts of protein. Imaging was initiated and continued for 2 to 3 hrs. Cross-sectional analysis was used to determine the dimensions of individual fibrinogen molecules. Due to the complexities associated with image enlargement, the height of the molecules was used as an initial indicator of molecular spreading.

Figure 1 illustrates a typical image for fibrinogen adsorbed on muscovite mica. A 1μm x 1μm image (Figure 1A, z-range = 8 nm) illustrates a field of both individual fibrinogen monomers and aggregates. The inset (1B) shows a single fibrinogen molecule with a characteristic trinodular structure. A cross-section (1C) clearly shows the three domains, labeled as D-E-D, and the measured heights of each of the domains. Figure 2 shows fibrinogen on HOPG in a similar manner. Again, a trinodular structure is observed, and cross-sectional analysis shows the height of the domains. Analysis of the molecules at sequential time points for each material results in a curve illustrating the time-dependence of the heights. This is seen in figure 3. For mica, the heights of both the D and E domains remain relatively constant over the time points studied. However, on HOPG, not only are the values of the measured heights ~½ that seen on mica, but they continue to decrease over the 2 hr time range. This is particularly notable for the D domains, and is consistent with what others have reported concerning the role of increased hydrophobicity of the D domain in fibrinogen film formation[2]. In summary, these results are consistent with extensive unfolding of adsorbed fibrinogen molecules on nonpolar hydrophobic surfaces as compared to polar hydrophilic materials, particularly in the relatively hydrophobic D domains[3].

References
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3. The authors would like to acknowledge financial support from the Whitaker Foundation.

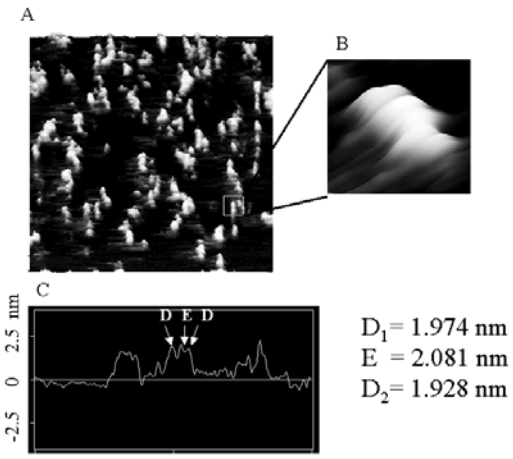
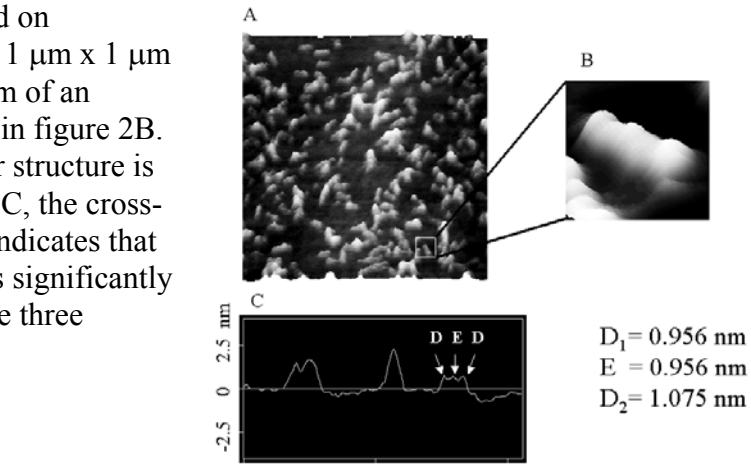


Figure 1: Fibrinogen molecules adsorbed on a hydrophilic mica substrate. Figure 1A is a 1 μm x 1 μm image, with a z-range of 8 nm. Figure 1B is a zoom of a single fibrinogen molecule as shown in the box in 1A. The trinodular structure is clearly visible. In 1C, a cross sectional analysis shows the three individual domains, labeled as D-E-D, as well as the measured heights of each.

Figure 2: Fibrinogen molecules adsorbed on hydrophobic HOPG. Figure 2A shows a 1 μm x 1 μm image, while a higher magnification zoom of an individual fibrinogen molecule is shown in figure 2B. As with the mica substrate, the trinodular structure is clearly visible in this protein. In figure 2C, the cross-section shows these three domains, and indicates that the height of the molecules on graphite is significantly less than that seen on mica for each of the three domains.



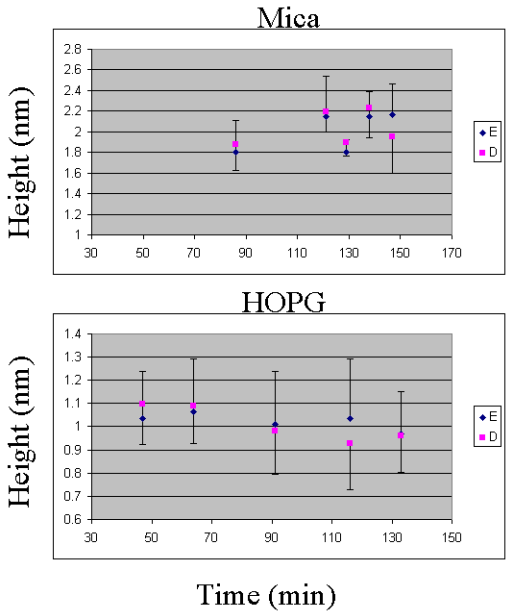


Figure 3: Time dependence of adsorbed fibrinogen height on muscovite mica and HOPG. On mica, the heights of both the D and E domains remain relatively constant and similar, while on HOPG, the height of the E domain remains relatively constant, but the height of the E domain decreases with time to a final value less than 1 nm. Note that both E and D domains are nearly ½ the height on HOPG as they are on mica. These results suggest that the relatively more hydrophobic D domain undergoes spreading to a greater degree than does the E domain, which may also be stabilized by the disulfide knot holding the six polypeptide chains together.

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