

AFM Characterization of Native and Mutant Fibrinogen

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ABSTRACT:

Dynamic mode atomic force microscopy was used to obtain high-resolution three-dimensional images of native and mutant fibrinogen molecules under dry conditions. Fibrinogen is a 340 kDa glycoprotein synthesized in liver hepatocytes and megakaryocytes. It plays a central role in the regulation of hemostasis and thrombosis, by participating in blood coagulation and facilitating adhesion and aggregation of platelets. Fibrinogen forms bridges between surface membrane proteins of platelets. Fibrinogen is a symmetrical dimer composed of six paired polypeptide chains, alpha, beta, and gamma (a, b, and g). On the alpha and beta chains, there is a small peptide sequence (called a fibrinopeptide). It is these small peptides that prevent fibrinogen from spontaneously forming polymers with itself. The N-terminal sections of these three chains are evolutionary related and contain cysteines that participate in the cross-linking of the chains. However, there is no similarity between the C-terminal part of the a chain and that of the b and g chains. The C-terminal part of the b and g chains forms a domain of about 270 amino-acid residues.¹

Dynamic mode atomic force microscopy ("tapping mode" or "intermittent contact") is capable of high resolution imaging without exerting constant destructive shear forces on the sample that may damage and smear subtle biological features. The exquisite vertical sensitivity of atomic force microscopy (<1 nm) allows for observation of critical membrane features. High-resolution images of native fibrinogen molecules were compared with mutant fibrinogen molecules after imaging. Use of the same imaging probe insured that the features had the same level of "erosion" (tip artifact) due to finite tip geometry. Image SXM was used to statistically analyze fibrinogen volumes indicating the mutant fibrinogen molecules were approximately 30% larger than the native structures.

¹ Hantgan RR, Francis CW, Marder VJ: Fibrinogen structure and physiology, in Colman RW, Hirsh J, Marder VJ, Salzman EW (eds): Hemostasis and Thrombosis: Basic Principles and Clinical Practice (ed 3). Philadelphia, PA, Lippincott, 1994