A Model of Mechanical Response of Individual Fibrin Fibers under Stress

Louise Jawerth
Harvard University
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Fibrin fiber networks are an important in the formation of blood clots. The viscoelastic properties of these networks are essential for their function. Predicting the behavior of these polymers in the bloodstream requires a model of their rheological behavior. As a first step towards this, we present a possible model for individual fibrin fibers undergoing stress. This model can be tested by comparison to single molecule pulling experiments data. We also present simulated force data for this comparison.

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I. INTRODUCTION:

Fibrinogen is a plasma protein important in homeostasis. It polymerizes into fibrin networks, the underlying structure of blood clots. The role of the clot is to stem the flow of blood during coagulation. This must be done under a large range of stresses and pressures. [1] In this article we examine the mechanical properties of fibrin fibers.

Fibrinogen is a 340 kDa dimer of two identical halves, each consisting of 3 peptide chains ($A\alpha$, $B\beta$, γ). Most of the molecular structure has been characterized by x-ray crystallography. The 3 peptide chains are organized into a central E domain connected via coiled coils to two outer globular D domains.[2] (see Fig. 1) Less well characterized are the αC domains of the γ-chain. It is currently thought that the αC domains protrude away from the D domain, arcing back towards the center of the molecule. [3].

Upon addition of thrombin, fibrinogen polymerize into fibrin protofibrils that aggregate into a fibrin fiber network. In normal physiological conditions, Factor XIII is also present in the blood stream. Factor XIII creates crosslinks between the fibrin monomers during polymerization. Crosslinking occurs between γ chains between D domains and between the αC domains of neighboring fibrinogen molecules.[4] (see Fig. 1 B)

Fibrin networks are the skeleton of a blood clot. During homeostasis the blood clot must stop the flow of blood under a variety of stresses and pressures. Changes in the mechanical properties of the network have been implicated in diseases such as myocardial infarction.[1] In fact, one current method for disease detection is thromobelastography, a technique that measures the viscoelastic properties of blood clots.[1]

The rheological properties of the overall network have been characterized with a combination of rheometers and scanning electron microscopy.[**?**] Recently, several groups have begun measuring the viscoelastic properties of individual fibrin fibers. They have shown individual crosslinked fibrin fibers have a breaking strain of around 250%. The fibers also have the remarkable property of being seemingly elastic to the point of breaking. (data not published) The next step in these measurements is to measure the force versus strain of an individual fibrin fiber.

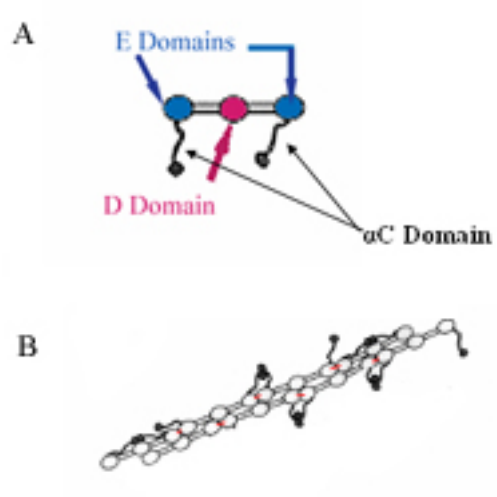

FIG. 1: Polymerization of a fibrin fiber from fibrinogen. (modified from [3]) A) shows a fibrinogen molecule with the central E domain in red and outer D domains in blue. The αC Domains are shown protruding from the D domains. B) shows a fibrin fiber. The αC are in black and the $\gamma - \gamma$ bonds are shown in red.

Although the fibrin network performs an important mechanical role, the origin of its mechanical properties is poorly understood. This is partially due to the lack of a good physical model of network mechanical response.[1] One step towards such a model is to create a model of the individual filaments that comprise the network. Presented within is a model of individual fibrin fibers under a stress parallel to the axis of the fiber.

II. MODEL

Fibrin fibers are highly elastic. In many biological molecules elasticity arises from highly disordered regions being straightened under stress and returning to a disordered state when the stress is removed. A similar mechanism could be responsible for the elasticity in fibrin fibers. During crosslinking the globular ends of the chain and the globular ends of the αC domain bind to two corresponding globular regions on another fibrinogen molecule forming $\gamma-\gamma$ dimers and α polymers. These two regions are likely candidates for the origin of elasticity.

To determine which of the globular regions is the origin of elasticity, we examine their contour lengths and compare to experiment. The γ region constitutes residues $146-406$ of the γ chain.[4] While not under stress, most of the length of the overall fibrinogen molecule arises from the long coiled coil regions. These correspond to about 150 amino acid residues. Fully outstretched, the $\gamma-\gamma$ dimers have a length of 260 amino acids. Therefore it seems unlikely that an outstretched $\gamma-\gamma$ globular region alone could be responsible for the 200% strain seen in experiment.

Following a similar analysis for the other globular region, the αC domain consists of a 170 residue "linker arm" and a 218 residue compact region.[3] The linker arm is a long floppy region that extends away from the coiled coils. Under stress, before unwinding the compact region the floppy region would have to straighten. Because the $\gamma-\gamma$ dimer is situated at the end of the coiled coils it is unlikely that the linker arm could straighten before stretching the $\gamma-\gamma$ dimer. This suggests a joint mechanism in which both regions are stretched.

For these reasons, a fibrin fiber undergoing stress we propose the following. Initially, as the fibrinogen fiber is undergoing stress, the $\gamma-\gamma$ regions start to stretch. Once the linker arms of the αC domain have straightened the compact region of the αC domain starts stretching. At some point, the $\gamma-\gamma$ regions will break. When this occurs, the αC domains alone will be stretched. (Fig. 2). The total contour length would then be consistent with the 200% strain seen in experiment.

1. *Modelling the System*

This system can be modelled as 2 parallel globular domains separated by flexible linkers. Previous work has modelled similar proteins with repeating *single* folded units. For instance, Titin was modelled as a series of globular regions separated by flexible linkers.[5] The titin model could be used to describe stretching of the $\gamma-\gamma$ dimers in fibrinogen, but would not account for the additional stretching of the αC domains. This model can be extended to include αC domains by adding an additional potential term.

In this system there are N monomers along the total length of the fibrin fiber. These are modelled as N independent globular regions connected by flexible linkers. The flexible linkers can be modelled as a simple spring with a slightly softened spring constant κ as described in [5] .

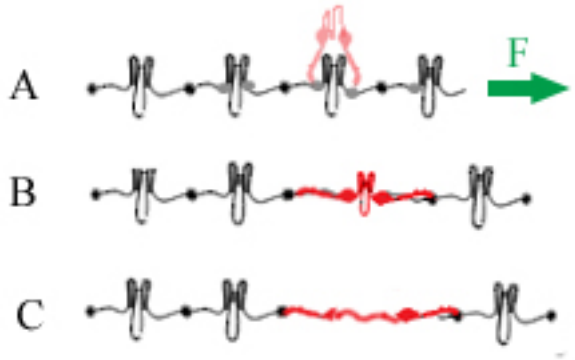

FIG. 2: A cartoon of a fibrin fiber under force (modified from [5]). The αC domain is shown in red. The αC domains on most units are not shown. A) shows the $\gamma-\gamma$ dimer being stretched. B) the linker arms are fulling extended and the globular part of the αC domain and the $\gamma-\gamma$ dimer are under tension. C) The αC domain alone is experiencing stress.

$$\beta V_{linker,i}(x) = \kappa\frac{x^2}{2} \tag{1}$$

In similar models globular regions unfolded sequentially. Each region was shown to behave according to the worm like chain (WLC) model until the extension reached a critical length when the folded domain would unravel.[6, 7] Similarly, we expect the $\gamma-\gamma$ dimers and αC domains to also be well-described by such a system.

$$-\beta l_p V_{WLC}(x) = \frac{x^2(3L-2x)}{4l_pL(L-x)} \tag{2}$$

where l_p is the persistence length and L is the total contour length.

With respect to the folded domains, for different values of $x(t)$ the potential represents one of the 4 different types of stretching as shown in Fig. 2. The total potential is either the potential from one, both or neither of the globular domains under stress. This behavior can be easily described by introducing a step function $\theta(x)$ into the equation,

$$\begin{aligned} V_{glob} \quad (x) = (1-\theta(x-x_\gamma))V_{WLC,\gamma}(x) + \\ \theta(x-l_a)(1-\theta(x-x_\alpha-l_a))V_{WLC,\alpha}(x-l_a) \end{aligned} \tag{3}$$

where l_a is the length of the αC domain linker arm, and $x_{\gamma,\alpha}$ are the critical lengths where the γ and αC globular regions unravel. [8]

The total potential along the fiber is the sum of the individual potentials.

$$V_{total}(x) \quad = \kappa \frac{x^2}{2} + \sum_{i=1}^{N} \sigma(r_i) V_{glob}(x - r_i) \qquad (4)$$

where,

$$\begin{aligned} \sigma(x,i) \quad &= \\ &\theta(x - (i-1)(l_a + x_\gamma)) \\ &(1 - \theta(x - (i+1)(l_a + x_\gamma + 1)) \end{aligned} \qquad (5)$$

The function $\sigma(x)$ is 1 for x in the interval between r_i and $r_i +$ the length of the linker arm. This was introduced to ensure the domains unfold sequentially.

2. Predicted Force Data

Several groups have recently started using optical tweezers and atomic force microscopy to stretch individual fibrin fibers and measure the corresponding force. Using equation (4) it is clear that our model predicts force data described by:

$$F_{total}(x) \quad = \kappa x + \sum_{i=1}^{N} \sigma(x,i) F_{glob}(x) \qquad (6)$$

where similar to above,

$$\begin{aligned} F_{glob}(x) \quad &= (1 - \theta(x - x_\gamma)) F_{WLC,\gamma}(x) + \\ &\theta(x - l_a)(1 - \theta(x - x_\alpha - l_a)) \\ &F_{WLC,\alpha}(x - l_a) \end{aligned} \qquad (7)$$

and,

$$F_{WLC}(x) \quad = \frac{1}{l_p}\{\frac{1}{4}(1 - \frac{x}{L})^{-2} - \frac{1}{4} + \frac{x}{L}\} \qquad (8)$$

Fig. 3 shows a corresponding force trace.

III. DISCUSSION

In this article, we have presented a possible model for the behavior of individual fibrin fibers undergoing a stress parallel to the axis of fiber in a network. This model was based on physical models of titin and other proteins under stress. These models were extended to include the affect of αC domains.

From experimental data we proposed a specific interaction between the αC domains and $\gamma - \gamma$ dimers. Previous models using single globular regions were extended to include the αC domains. Semi-flexible linker were modelled as springs and both globular regions were described by a WLC chain model.

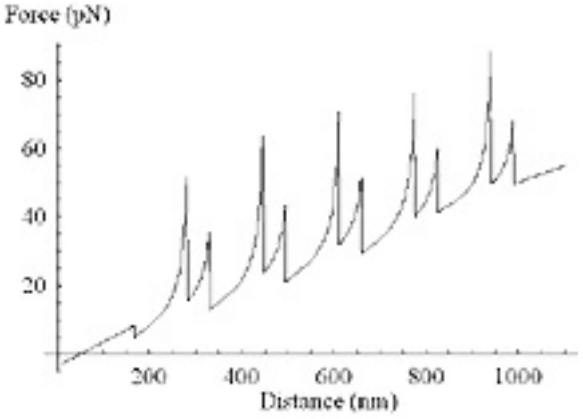

FIG. 3: Simulated force data predicted for a single molecule pulling experiment. With the following constants $\kappa = .05pN/nm$, $l_a = 170a$, $l_p = .4$ nm, $N = 4$ $x_\alpha = 325a$, and $x_\gamma = 350a$ and $a = 1/3nm$ (an estimate for number of amino acids per nanometer)

The best test for the validity of this model will be through comparison with single molecule pulling experiments data. Several groups are currently taking these measurements using optical tweezers and atomic force microscopy. These experiments will measure the force versus extension from a fibrin fiber under stress. When this force data is available it can be compared to the force data predicted by this model. In particular we expect to see a characteristic double peak in the force data. For the simulated force trace in Fig. 3 values for the persistence length, spring constant and contour lengths of the αC and γ regions were assumed. Experimental data will provide exact values for these constants. Once these values have been obtained the model can be developed to predict overall network behavior.

In conclusion, fibrin fiber networks play an important mechanical role in blood coagulations. To fully understand their function we must develop a model that predicts their viscoelastic behavior. In this paper, we have presented a model for individual fibrin fiber response to a mechanical stress along the axis of the fiber. Validity of this model will be through comparison of future force versus strain data obtained in single molecule fibrin pulling experiments.

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[8] Estimates for the critical lengths can be obtained by modelling the folding and unfolding of a single region as a 2 state system. The critical length, x_u can be estimated from the probability dP_u of unfolding where $dP_u = N\alpha_0 e^{\frac{Fx_u}{(k_B T)}}\Delta t$ where $\alpha_0 = \omega e^{\frac{-\Delta G_u}{(k_B T)}}$, and G_u is the activation barrier of the unfolding process. details given in [5]