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Length of Tandem Repeats in Fibrin's αC Region Correlates with Fiber Extensibility

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The mechanical properties of blood clots are of central importance to hemostasis, thrombosis and embolism. Fibrin fiber networks are the major structural constituent of clots, and numerous studies dating back several decades have characterized their macroscopic viscoelastic properties [1,2]. The fiber-level and molecular details giving rise to these properties have not been established, however. The correlation between mechanical properties and amino acid sequence is critical for a predictive understanding of the role of genetic defects in clot pathologies. To address this issue, we have developed a nanomanipulation technique for evaluating individual fibrin fibers. It consists of a combination fluorescence/atomic force microscope system that permits viewing of fiber deformation simultaneous with quantitative strain data. Recently we and our colleagues reported on the high extensibility of individual human fibrin fibers [3], with extensibility (or strain at breaking) of some fibers exceeding 300%, and elastic recovery with strains of up to 180%. This places human fibrin among the most extensible protein polymers, exceeding elastin and resilin in extensibility [4]. Here we test the hypothesis that the majority of the strain is taken up by the tandem repeat segment of the flexible αC region of fibrin. Our study focused on this portion of the protein by mechanically evaluating fibrins with varying lengths of the tandem repeat segment. Using our integrated nanomanipulation system, we stretched individual fibrin fibers made of human, mouse and chicken fibrinogen which have long, intermediate and zero length tandem repeat segments respectively. We found that extensibility correlated with the lengths of the tandem repeat segments.

The fibrin monomer is 45 nm long and consists of three pairs of polypeptides (α, β, γ) joined through over two dozen disulfide bridges (for review see [5]) . The monomer has two globular regions (D) on the ends of the molecule joined to a smaller central globular region (E) through a triple coiled-coil (Fig. 1A). The crystal structure for the majority of the fibrinogen molecule has been solved [6]. However, roughly 2/3 of the α chain on the C-terminal end known as the "αC region" (α221-610 in human), has defied definitive structural characterization. These αC regions have been shown to interact and to facilitate fiber formation, and have been implicated in the mechanical performance of clots [7,8]. What has received less attention is the question of how this portion of the fibrin molecule operates as a mechanical element. The αC region has two distinct parts: the connector region (α221-391 in human) and the terminal region (α392-610 in human). Within the connector region are a series of tandem amino acid repeats.

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Both the number of repeats and the number of residues within the repeat differ greatly between species [9,10]. We took advantage of this inherent variability to compare the extensibility of fibers with no tandem repeat segment (chicken), intermediate length (mouse) and long (human) repeat segment (Figure 1B). In human fibrinogen, the tandem repeat segment is 128 amino acids long (10 repeats of mostly 13), in mouse 60 (5 repeats of mostly 13) [10], and in chicken fibrinogen, is completely absent. Consequently, the contour length of the loosely tethered αC region is roughly 22 nm longer in mouse and 50 nm longer in human than in chicken fibrinogen. With two α chains per monomer, this would be a total difference in contour length of 44 nm and 100 nm per monomer.

To test the extensibility of individual fibrin fibers, we polymerized low concentrations of fibrinogen onto micropatterened corrugated surfaces of transparent ridges and channels such that fibrin fibers were suspended across the channels [3,4]. We then used the combined AFM-fluorescence microscope to stretch the fibers to the point of failure while simultaneously monitoring deformation through video microscopy. Video of the extension was recorded for each pull, and analyzed to determine extensibility. The extensibility is simply the change in length of the fiber segment at the breaking point, divided by the original length (here presented as a percentage). An example stretching sequence is shown in panels C-F of Fig. 1. Our extensibility results are shown in panels G and H of Fig. 1. Extensibility for chicken fibers is 47 +/- 23 % (N=42); mouse: 187 +/- 44 % (N=89); human: 217 +/- 47 (N=75). The differences were significant for each pairing (mouse/human, $p=0.0004$; chicken/human, $p < 0.0001$; mouse/chicken, $p< 0.0001$). Thus the relatively low extensibility of chicken fibrin, and the intermediate values for mouse as compared to human fibers, correlate with the length of their corresponding connector regions.

Along with the mechanical evidence, the primary structure of the repeat segment also suggests mechanical function. The segment is intriguingly reminiscent of repeat sequences in elastomeric proteins such as elastin, resilin, and spider-silk [11]. Though the sequence of the repeats varies in length and content among these proteins, they share some interesting similarities such an unusually high content of glycine and proline (21% and 11% respectively in human fibrin). These amino acids have been implicated as blockers of secondary structure and in high frequency promote amorphous or “natively unfolded” structure [12-14]. Natively unfolded protein domains have been shown to function as non-linear springs [15] as well as providing tethers for multiple interactions facilitated by lack of strict conformational constraints [16].

Fibrin is a very large protein (340 kDa) and has other domains that are candidates for conformational changes under strain. These include the two globular D regions at each end of the protein (the C terminal of the β and γ chains), and the coiled coil region. A recent force spectroscopy study on engineered oligomers of fibrinogen demonstrated that the unfolding of the coiled- coil region could account for a fraction of fibrin’s extensibility (up to strains of 100%) [17], while at the same time found no evidence for contribution from the globular D regions. Because that study used engineered, end-to-end linked oligomers, the contribution of the αC region was not addressed. Aside from the variant tandem repeat segment, there are other substantive differences in the primary structures of the three fibrins studied here, and these may influence the species-specific properties. Nevertheless, the similarity of the primary structure of the tandem repeat segment of the αC with other elastomeric proteins suggests it exhibits similar mechanical properties and may have a significant role in the extensibility of fibrin fibers, and ultimately the mechanics of fibrin clots.

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References

1. Gerth C, Roberts WW, Ferry JD. Rheology of fibrin clots. II. Linear viscoelastic behavior in shear creep. Biophys Chem 1974;2:208–17. [PubMed: 4474029]
2. Ryan EA, Mockros LF, Weisel JW, Lorand L. Structural origins of fibrin clot rheology. Biophys J 1999;77:2813–26. [PubMed: 10545379]
3. Liu W, Jawerth LM, Sparks EA, Falvo MR, Hantgan RR, Superfine R, Lord ST, Guthold M. Fibrin fibers have extraordinary extensibility and elasticity. Science 2006;313:634. [PubMed: 16888133]
4. Guthold M, Liu W, Sparks EA, Jawerth LM, Peng L, Falvo M, Superfine R, Hantgan RR, Lord ST. A comparison of the mechanical and structural properties of fibrin fibers with other protein fibers. Cell Biochem Biophys 2007;49:165–81. [PubMed: 17952642]
5. Weisel JW. Fibrinogen and fibrin. Adv Protein Chem 2005;70:247–99. [PubMed: 15837518]
6. Yang Z, Kollman JM, Pandi L, Doolittle RF. Crystal structure of native chicken fibrinogen at 2.7 A resolution. Biochemistry 2001;40:12515–23. [PubMed: 11601975]
7. Litvinov RI, Yakovlev S, Tsurupa G, Gorkun OV, Medved L, Weisel JW. Direct Evidence for Specific Interactions of the Fibrinogen alphaC-Domains with the Central E Region and with Each Other. Biochemistry 2007;46:9133–42. [PubMed: 17630702]
8. Collet JP, Moen JL, Veklich YI, Gorkun OV, Lord ST, Montalescot G, Weisel JW. The alphaC domains of fibrinogen affect the structure of the fibrin clot, its physical properties, and its susceptibility to fibrinolysis. Blood 2005;106:3824–30. [PubMed: 16091450]
9. Wang YZ, Patterson J, Gray JE, Yu C, Cottrell BA, Shimizu A, Graham D, Riley M, Doolittle RF. Complete sequence of the lamprey fibrinogen alpha chain. Biochemistry 1989;28:9801–6. [PubMed: 2611265]
10. Murakawa M, Okamura T, Kamura T, Shibuya T, Harada M, Niho Y. Diversity of primary structures of the carboxy-terminal regions of mammalian fibrinogen A alpha-chains. Characterization of the partial nucleotide and deduced amino acid sequences in five mammalian species; rhesus monkey, pig, dog, mouse and Syrian hamster. Thromb Haemost 1993;69:351–60. [PubMed: 8497848]
11. Tatham AS, Shewry PR. Comparative structures and properties of elastic proteins. Philos Trans R Soc Lond B Biol Sci 2002;357:229–34. [PubMed: 11911780]
12. Rauscher S, Baud S, Miao M, Keeley FW, Pomes R. Proline and glycine control protein self-organization into elastomeric or amyloid fibrils. Structure 2006;14:1667–76. [PubMed: 17098192]
13. Crasto CJ, Feng J. Sequence codes for extended conformation: a neighbor-dependent sequence analysis of loops in proteins. Proteins 2001;42:399–413. [PubMed: 11151011]
14. Doolittle RF, Kollman JM. Natively unfolded regions of the vertebrate fibrinogen molecule. Proteins 2006;63:391–7. [PubMed: 16288455]
15. Linke WA, Kulke M, Li H, Fujita-Becker S, Neagoe C, Manstein DJ, Gautel M, Fernandez JM. PEVK domain of titin: an entropic spring with actin-binding properties. J Struct Biol 2002;137:194–205. [PubMed: 12064946]
16. Dunker AK, Brown CJ, Lawson JD, Iakoucheva LM, Obradovic Z. Intrinsic disorder and protein function. Biochemistry 2002;41:6573–82. [PubMed: 12022860]
17. Brown AE, Litvinov RI, Discher DE, Weisel JW. Forced Unfolding of Coiled-Coils in Fibrinogen by Single-Molecule AFM. Biophys J 2007;92:L39–41. [PubMed: 17172299]

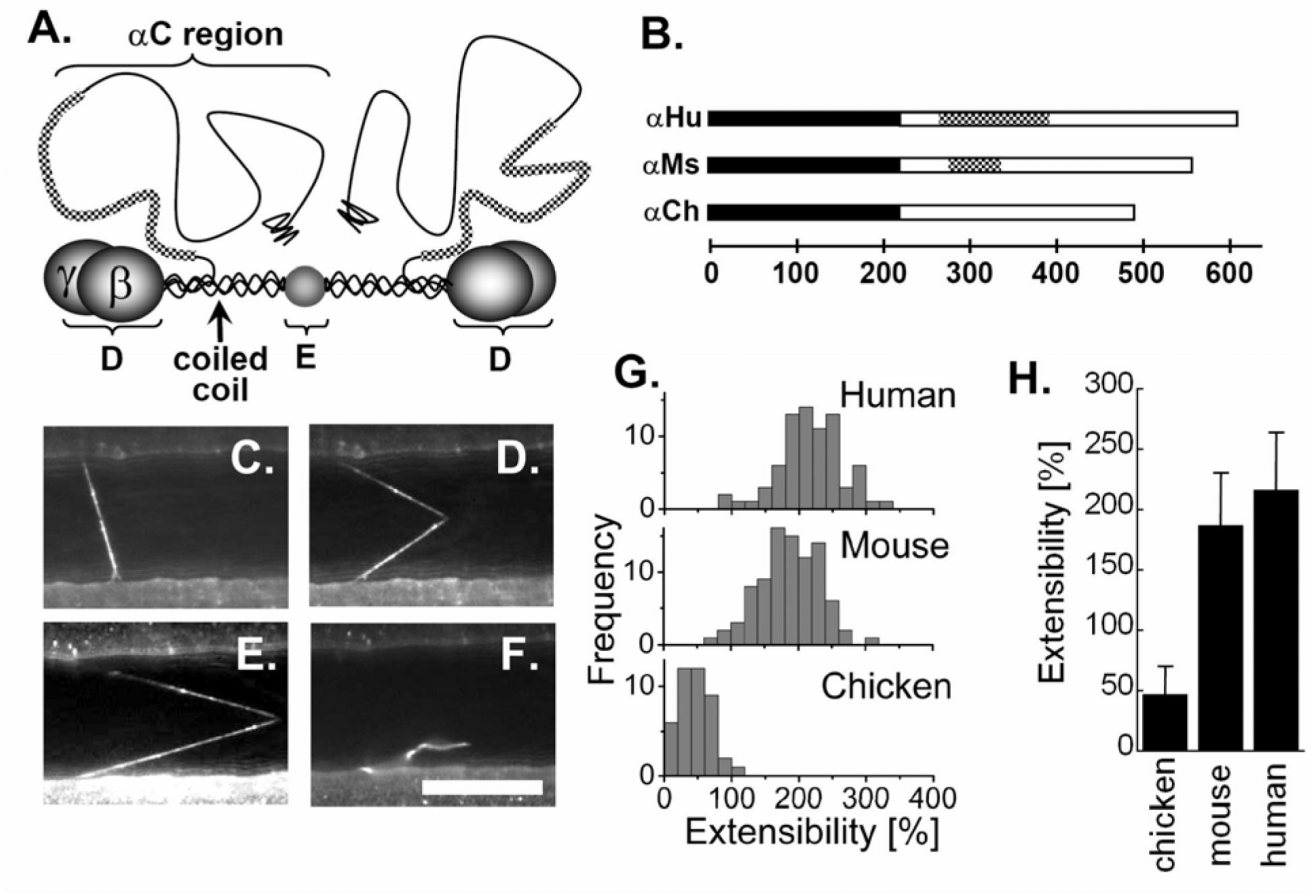

Figure 1.

(A) Cartoon of fibrin molecule depicting released αC region. The thicker checked portion indicates the approximate location of the tandem repeat segment. (B) Schematics of the α chain for human, mouse and chicken. Solid black indicates the coiled coil region, white indicates the αC region and the repeat segment of the αC is overlaid with a checked pattern. (CE) Fluorescence images of suspended human fibrin fiber stretched to 230% strain with the AFM tip (not visible). Channel/Ridge structures were prepared through microcontact printing using a patterned silicone rubber stamp and a UV curable adhesive to produce 25 micron channels 10 microns deep [3,4]. Human and mouse fibrinogens, human factor XIII, human and mouse thrombin were purchased from Enzyme Research Laboratories, Inc. Chicken fibrinogen was purified from fresh-frozen chicken plasma. Human thrombin was used to prepare clots from human and chicken fibrinogens; mouse thrombin was used with mouse fibrinogen. Clots prepared under the conditions used for microscopy were analyzed by SDS-PAGE; all showed substantive crosslinking, as identified by gamma-gamma dimers. Final concentration of reagents were 0.02 mg/mL fibrinogen, 0.1 U/mL thrombin, 0.05 μg/mL Factor XIII in 5 mM calcium HBS. Fibrinogen was fluorescently labeled after polymerization with 24nm volume-labeled red fluorescent carboxyl coated microspheres. The fiber is suspended across a channel and manipulated with the AFM tip (not visible). All figures are at same scale; scale bar = 20μm. (G) Histograms of extensibility measurements. (H) Bar graph of averages of the data depicted in the histograms. Error bars represent standard deviations.