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John Ferry and the mechanical properties of cross-linked fibrin

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Abstract

This article describes the role John Ferry played in relating the location of cross-linked γ-chains in fibrin fibrils to the mechanical properties of fibrin clot.

Keywords: Fibrin; Cross-linking; Factor XIII; John Ferry

Fibrin assembly begins after thrombin cleavage of fibrinopeptide A from the E domain of fibrinogen. This event exposes a polymerization site termed E_A, which then combines with a constitutive complementary site in the D domain ("Da") of a neighboring molecule to initiate the fibrin assembly process (Fig. 1). These noncovalent intermolecular interactions (labeled "D:E" in the diagram) govern fibrin assembly, and result in a half-staggered overlapping double-stranded arrangement with periodicity corresponding to one-half the length of a fibrinogen molecule (22.5 nm), as first proposed by Ferry [1]. D:E interactions also facilitate antiparallel alignment of opposing C-terminal γ-chain pairs, thereby accelerating factor XIIIa-mediated cross-linking to form γ-dimers (γ–γ). In a companion article in this volume, I paid tribute to John Ferry's contribution to this subject [2]. In this article, I want to describe a final contribution that related the mechanical properties of γ-chain cross-linked fibrin to the location of the cross-linked γ-chains in the polymer structure.

During the past 20 years, there has been, to say the least, a vigorous exchange concerning the location of cross-linked C-terminal γ-chains within assembled fibrin fibrils. Specifically, the issue concerns whether cross-linked γ-chains are positioned transversely between each strand of a fibril (Fig. 1, panel A) or, alternatively, longitudinally ("DD-long" in the older terminology) along each strand of a fibril (panel B). Summary arguments for the different cross-linking arrangements have recently been published in the "Debate" section of the *Journal of Thrombosis and Haemostasis* [3,4]. Each article has argued either for one viewpoint or the other, but never for both. I and a few of my colleagues

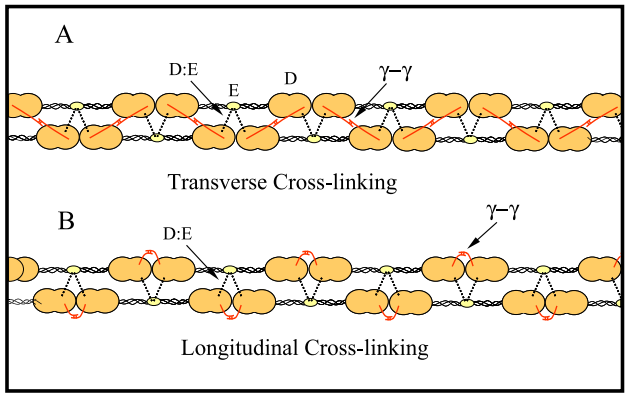

Fig. 1. Alternative γ-chain cross-linking patterns for fibrin fibrils. Panel A, transverse; panel B, longitudinal. When fibrin units (D domains, orange; E domain, yellow) assemble to form a double-stranded fibril due to noncovalent intermolecular 'D:E' interactions (dotted lines), the C-terminal γ-chain regions become aligned in an antiparallel arrangement. They are subsequently covalently cross-linked by factor XIIIa to form γ-dimers (γ–γ, in red). The dispute at hand is whether cross-linked γ-chains are situated 'transversely' between fibril strands (panel A) or 'longitudinally' along each fibril strand (panel B).

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Fig. 2. A picture taken at one of our group meetings. Left side from the bottom up: David Meh, John Ferry, Michael Mosesson, Yves Delmotte; right side from the top, Kevin Siebenlist, Jim DiOrio.

and associates (including John Ferry, as will be told) have opted for "transverse" positioning, whereas notable and respected investigators like John Weisel and Russell Doolittle have argued for "longitudinal" positioning. I write this present article not to proselytize, but rather to use the context of the debate article to cite a significant contribution by John D. Ferry, whose perspicacity on the matter of γ-chain positioning transformed a controversy of uncertain physiological significance into one directly related to the viscoelastic behavior of fibrin.

After his retirement as Chairman of the Chemistry Department in 1982, and almost until his death [5], Ferry maintained an active interest in fibrin assembly and structure by actively communicating with investigators in the field, including person-to-person meetings and discussions. From his retirement onward, including a vivid

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March 11, 1996 **FAX # 608-262-0381**

Michael W. Mosesson, M.D.
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Dear Mike:

I want to thank you again for inviting me to attend the hemostasis meeting last month. I enjoyed very much renewing associations with the research groups and hearing your review of your recent ingenious experiments, reprints of which you had sent me earlier.

The question came up in the discussion as to the implications of DD-long vs. DD-transverse on mechanical properties. We are hampered by lack of any real theory connecting elasticity with structure. However, it occurred to me that DD-transverse has the possibility of a large extension under tensile stress if the D:E non-covalent bonds (and the D:D interactions discussed in your paper on Tokyo II) can yield under tensile stress. The enclosed sketch indicates what might happen to the lower diagram of Fig. 1, p. 89 of your paper in *J. Struct. Biol.* if the double-stranded fiber opened up to support tension only through a sequence of covalent-bond-linked units. This would increase the length by a factor of about 1.8. Some lability of the D:E is indicated by the fact that unligated clots creep over long times even under low stresses and at large shearing stresses there are substantial modifications of structure, both of these effects being climinated by ligation (cross-linking).

Although a clot can undergo a fairly large shearing strain it cannot be stretched in elongation much without breaking. Anyway, the relation between macroscopic stretch and fibril elongation is complicated by orientation of the fibers. Fibrin film is tougher and can be cut in strips and stretched, although the relation of macroscopic stretch to fibril extension again would not be simple. We found that stretching fibrin film caused an apparent increase in x-ray spacing by a factor of 1.28 independent of the macroscopic stretch (Table I of Paper II enclosed, p. 1841). This has never been explained – the evidence is a little fuzzy and it may be a red berring.

Cross-linked (ligated) fibrin film recovers its original length after a stretch by a factor of 1.82 (Fig. 6 of Paper I enclosed). This macroscopic stretch has been attributed to the bending and orientation of fibers. I am curious as the extension and recovery of such a film after soaking it in a GPRP solution to dissociate the non-covalent bonding. The fibrils might open up as in the enclosed sketch and would be unlikely to recover, leaving a permanent deformation. Unfortunately, however, the interpretation of results of such an experiment would be far from definitive. I wish I could offer some better thoughts.

With very best regards,

Sincerely yours,

John D. Ferry

Enclosures

Fig. 3. John Ferry's letter dated March 11, 1996.

keynote presentation at a 1982 New York Academy of Sciences meeting [6], we frequently met with him over a long lunch or during an evening to present and discuss our studies (Fig. 2). At one such meeting, we presented studies [7,8] concerned with the issue of transverse versus longitudinal XIIIa-mediated fibrin cross-linking. The discussion on that occasion was stimulating, and was particularly focused on the potential relationship between the two cross-linking arrangements and the known mechanical properties of a fibrin clot. A few days after our meeting, I received a letter containing John Ferry's carefully reasoned thoughts on this matter (Fig. 3). This letter included an interpretation of the potential relationship between transversely cross-linked γ-chains and the effect of their presence on the mechanical properties of fibrin. He also enclosed a sketch (Fig. 4, upper) about which he wrote, "The enclosed sketch indicates what might happen... if the double-stranded fiber opened up to support tension only through a sequence of covalent-bond-linked units. This would increase the length by a factor of about 1.8." He connected this idea to measurements that he and his colleagues had made on stretched fibrin films [9,10]. Their data indicated that a cross-linked fibrin film could be stretched 1.8-fold beyond its original length before breaking, and also that it could completely recover its original form from this maximally stretched position.

With this incredibly insightful interpretation, John Ferry had forged an important link between the mechanical properties of fibrin and the transverse positioning of cross-linked γ-chains (Fig. 1, panel A). The significance of his conjecture did not immediately dawn upon me. However, after several rereadings of this letter, I finally realized that only γ-chains that were transversely positioned and cross-linked could account for the viscoelastic properties of a stretched fibrin film. By contrast, "longitudinal" cross-linking (Fig. 1, panel B) could not possibly account for these mechanical properties, since their positioning along each fibril strand would place no constraints upon the degree of polymer elongation that could take place, nor could they conceivably contribute to elastic recovery of a maximally stretched fibrin film. The invitation by the *Journal of Thrombosis and Haemostasis* to formally debate John Weisel on the subject of γ-chain cross-linking afforded me an unusual opportunity to critically discuss each of the experimental arguments. Thanks to John Ferry's letter, I was able to place these arguments into physiological perspective (Fig. 4). His many contributions to the field of polymer chemistry are well recognized, but none was more

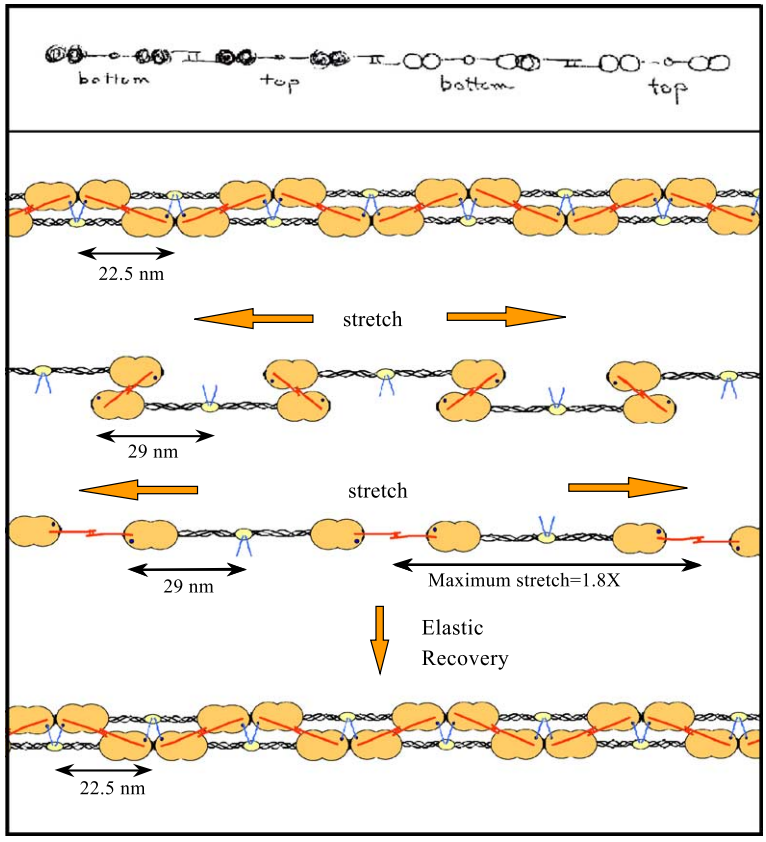

Fig. 4. A diagram that graphically presents what probably takes place when a transversely cross-linked fibrin film is stretched. Ferry's original sketch is reproduced in the upper portion of the figure. "Top" and "bottom" refer to the upper and lower strands, respectively, of the previously unstretched double-stranded fibril. The diagram is adapted from Ref. [3].

important, at least to me, than this contribution to our understanding of the mechanical behavior of fibrin.

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