

Fibrinogen and fibrin structure and functions

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Summary. Fibrinogen molecules are comprised of two sets of disulfide-bridged A α -, B β -, and γ -chains. Each molecule contains two outer D domains connected to a central E domain by a coiled-coil segment. Fibrin is formed after thrombin cleavage of fibrinopeptide A (FPA) from fibrinogen A α -chains, thus initiating fibrin polymerization. Double-stranded fibrils form through end-to-middle domain (D:E) associations, and concomitant lateral fibril associations and branching create a clot network. Fibrin assembly facilitates intermolecular anti-parallel C-terminal alignment of γ -chain pairs, which are then covalently ‘cross-linked’ by factor XIII (‘plasma protransglutaminase’) or XIIIa to form ‘ γ -dimers’. In addition to its primary role of providing scaffolding for the intravascular thrombus and also accounting for important clot viscoelastic properties, fibrin(ogen) participates in other biologic functions involving unique binding sites, some of which become exposed as a consequence of fibrin formation. This review provides details about fibrinogen and fibrin structure, and correlates this information with biological functions that include: (i) suppression of plasma factor XIII-mediated cross-linking activity in blood by binding the factor XIII A₂B₂ complex. (ii) Non-substrate thrombin binding to fibrin, termed antithrombin I (AT-I), which down-regulates thrombin generation in clotting blood. (iii) Tissue-type plasminogen activator (tPA)-stimulated plasminogen activation by fibrin that results from formation of a ternary tPA-plasminogen-fibrin complex. Binding of inhibitors such as α_2 -antiplasmin, plasminogen activator inhibitor-2, lipoprotein(a), or histidine-rich glycoprotein, impairs plasminogen activation. (iv) Enhanced interactions with the extracellular matrix by binding of fibronectin to fibrin(ogen). (v) Molecular and cellular interactions of fibrin β 15–42. This sequence binds to heparin and mediates platelet and endothelial cell spreading, fibroblast proliferation, and capillary tube formation. Interactions between β 15–42 and vascular endothelial (VE)-cadherin, an endothelial cell receptor, also promote capillary tube formation and angiogenesis. These activities are enhanced by binding of growth factors like fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor (VEGF), and cytokines like interleukin (IL)-1. (vi) Fibrinogen

binding to the platelet $\alpha_{IIb}\beta_3$ receptor, which is important for incorporating platelets into a developing thrombus. (vii) Leukocyte binding to fibrin(ogen) via integrin $\alpha_M\beta_2$ (Mac-1), which is a high affinity receptor on stimulated monocytes and neutrophils.

Keywords: endothelial cells, extracellular matrix, fibrin, fibrinolysis, growth factors, leukocytes.

Introduction

Fibrinogen and fibrin play overlapping roles in blood clotting, fibrinolysis, cellular and matrix interactions, the inflammatory response, wound healing, and neoplasia. These functions are regulated by interactive sites on fibrin(ogen), some of which are masked or otherwise not available on fibrinogen, and they commonly evolve as a consequence of fibrin formation or fibrinogen–surface interactions. The present article relates structural features of fibrin(ogen) and fibrin polymerization and cross-linking, with its multiple biologic functions, including binding to thrombin, fibrinolysis, regulation of factor XIII activity, growth factor binding, and interactions with cells including platelets, leukocytes, fibroblasts, and endothelial cells.

Fibrinogen structure, conversion to fibrin, and fibrin assembly

Fibrinogen molecules are elongated 45 nm structures that consist of two outer D domains, each connected by a coiled-coil segment to its central E domain (Fig. 1). The molecule is comprised of two sets of three polypeptide chains termed A α , B β , and γ , which are joined together in the N-terminal E domain by five symmetrical disulfide bridges [1–5]. Non-symmetrical disulfide bridges form a ‘disulfide ring’ in this region [2,4].

The A α -chain consists of 610, the B β -chain 461, and the major γ -chain form, γ_A , 411 residues [1]. A minor γ -chain variant termed γ' , arises through alternative processing of the primary mRNA transcript [6], resulting in substitution of γ_A 408–411V with a unique anionic 20 amino acid sequence ($\gamma'408$ –427L) that contains two sulfated tyrosines [7,8]. γ' -Chains account for approximately 8% of the total fibrinogen γ -chain population and are mainly found in heterodimeric fibrinogen molecules amounting to approximately 15% of plasma fibrinogen molecules [9]. Homodimeric γ'/γ' -molecules

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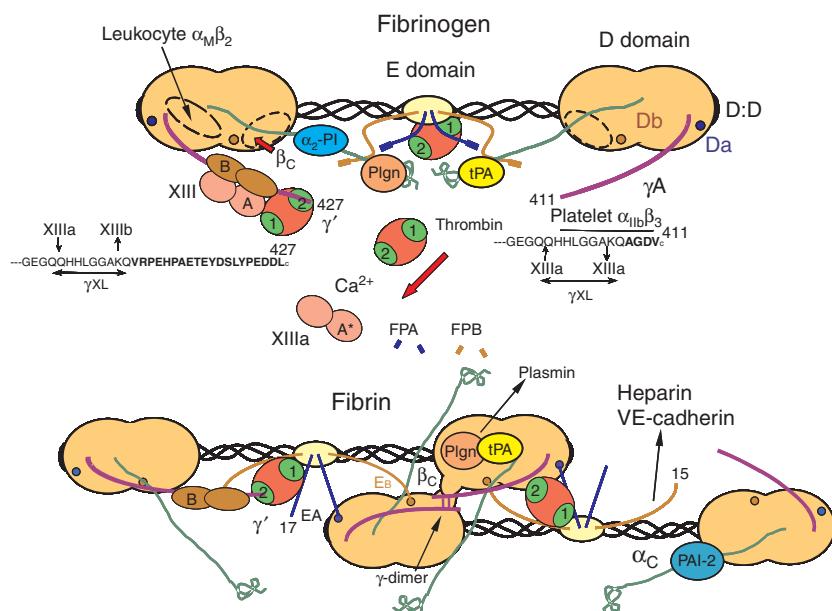


Fig. 1. Schematic diagram of fibrinogen structure, its conversion to fibrin, and the thrombin-mediated conversion of native factor XIII to XIIIa. Binding sites for proteins, enzymes, receptors, and other molecules that participate in fibrin(ogen) functions are illustrated.

account for <1% of the circulating fibrinogen molecules in blood [10].

Each fibrinogen α -chain contains an N-terminal fibrinopeptide A (FPA) sequence, cleavage of which by thrombin initiates fibrin assembly [11–13] by exposing a polymerization site termed E_A . One portion of E_A is at the N-terminus of the fibrin α -chain comprising residues 17–20 gly-pro-arg-val (GPRV) [14], and another portion is located in the fibrin β -chain between residues 15 and 42 [15–18]. Each E_A -site

combines with a constitutive complementary-binding pocket (Da) in the D domain of neighboring molecules that is located between γ 337 and γ 379 [18–20]. The initial E_A :Da associations cause fibrin molecules to align in a staggered overlapping end-to-middle domain arrangement to form double-stranded twisting fibrils [21–24] (Fig. 2). Fibrils also undergo lateral associations to create multi-stranded fibers [25,26].

Two types of branch junctions occur in fibrin networks [27]. The first occurs when a double-stranded fibril converges

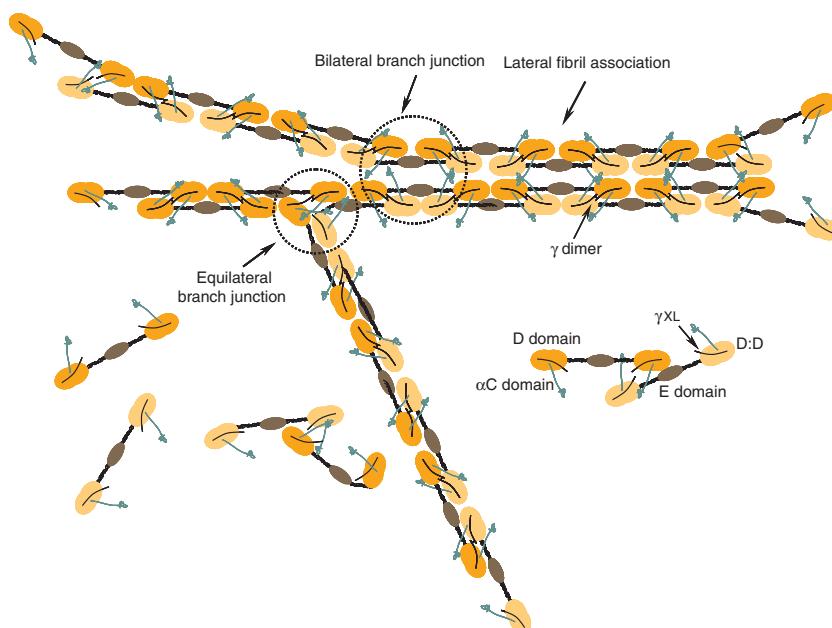


Fig. 2. Schematic diagram of fibrin assembly, branching, lateral fibril association, and γ -chain cross-linking. Fibrin molecules are represented in two color schemes for ease of recognition. Cross-linked γ -chains are positioned ‘transversely’ between fibril strands, as discussed in text (adapted from Mosesson [184]).

laterally with another fibril to form a four-stranded fibril, a so-called 'bilateral' junction. Lateral convergence of additional fibrils results in multi-stranded versions of this structure. The second type of branch junction, termed 'equilateral', is formed by convergent interactions among three fibrin molecules that give rise to three double-stranded fibrils. Equilateral junctions form with greater frequency when fibrinopeptide cleavage is relatively slow [28], and under such conditions, the networks are more branched and the matrix is 'tighter' (i.e. less porous) than those formed at high levels of thrombin [29].

Release of fibrinopeptide B (FPB; B β 1–14) is slower than release of FPA [11–13]. The process exposes an independent polymerization site, E_B [30], beginning with β 15–18 gly-his-arg-pro (GHRP) [14] that interacts with a constitutive complementary Db site in the β -chain segment of the D domain [20,31]. This interaction contributes to lateral association by inducing rearrangements in the β_C -region of the D domain that promote intermolecular $\beta_C:\beta_C$ contacts [32], as illustrated in Fig. 1. Polymerization of des-BB fibrin results in the same type of fibril structure as occurs with des-AA fibrin [28], but the clot strength is lower than that of des-AA fibrin [30].

The ' α C'-domain originates at residue 220 in the D domain, not far from where it emerges from the D domain, and it terminates at A α 610 [33]. Fibrin clots formed from fibrinogen 'catabolite' fractions I-6–I-9, which lack C-terminal portions of α C-domains, display prolonged thrombin times, reduced turbidity, and generate thinner fibers [34–36]. In fibrinogen, α C-domains tend to be non-covalently tethered to the E domain [37–39], but dissociate from it following FPB cleavage [38,39]. This event evidently makes α C-domains available for interaction with other α C-domains, thereby promoting lateral fibril associations and more extensive network assembly.

There are two constitutive self-association sites in the γ -chain region of each D domain (the ' γ -module') that participate in fibrin or fibrinogen assembly and cross-linking, namely ' γ_{XL} ' and 'D:D' [40,41]. The γ_{XL} -site overlaps the γ -chain cross-linking site (Fig. 1). Intermolecular association between two γ_{XL} -sites promotes alignment of cross-linking regions for subsequent factor XIII- or FXIIIa-mediated transglutamination [40,42,43]. Each 'D:D' site is situated at the outer portion of a fibrin(ogen) D domain between residues 275 and 300 of the γ -module [44]. These sites are necessary for proper end-to-end alignment of fibrinogen or fibrin molecules in assembling polymers. Congenital dysfibrinogenemic molecules such as fibrinogen Tokyo II (γ R275C) [41], which have defective 'D:D' site interactions, are characterized by networks displaying increased fiber branching that evidently results from slowed fibrin assembly, plus inaccurate end-to-end positioning of assembling fibrin monomers.

Fibrin cross-linking and the viscoelastic properties of fibrin

The C-terminal region of each fibrinogen or fibrin γ -chain contains one cross-linking site at which factor XIII or XIIIa catalyzes the formation of γ -dimers [40,42,45,46] by introducing reciprocal intermolecular ϵ -(γ -glutamyl)lysine covalent bonds

between the γ 406 lysine of one γ -chain and a glutamine at γ 398/399 of another (cf. Fig. 1) [47–49]. The same type of intermolecular ϵ -(γ -glutamyl)lysine bridging occurs more slowly among amine donor and lysine acceptor sites in A α - or α -chains [50,51], thereby creating α -oligomers and larger α -polymers [42,45,52]. Cross-linking also occurs among α - and γ -chains [25,53], and intramolecular cross-linked α - γ -chain heterodimers have been identified in plasma fibrinogen molecules [54].

The Da:E_A interaction, which drives fibrin assembly, facilitates intermolecular antiparallel alignment of γ -chain pairs at γ_{XL} -sites, thereby accelerating the cross-linking rate [40,43,46,55]. At physiologic concentrations, alignment of γ -chains is slower in fibrinogen than it is in fibrin, and therefore cross-linking takes place more slowly in fibrinogen. However, since the fibrinogen cross-linking rate is strongly concentration-dependent, the fibrinogen cross-linking rate can be accelerated even beyond that of fibrin simply by raising the fibrinogen concentration [46].

The precise location of cross-linked γ -chains in assembled fibrin fibrils has been a controversial issue for many years. Whether these chains are positioned 'transversely' between fibril strands (cf. Figs 1–3), or 'longitudinally' along each strand of a fibril, has been rigorously debated but not yet decided to everyone's satisfaction [56–59]. In this study, I have assumed that, the cross-linked γ -chains in fibrin fibrils are located 'transversely' between the D domains of opposing strands of a cross-linked fibrin fibril, a biased but nevertheless carefully reasoned view. The reader wishing to gain a better perspective of the differing views should consult the debate articles themselves. One reason for representing cross-linking as transverse is that this is the only cross-linking arrangement that can account for an important viscoelastic property of fibrin, namely that after a maximum stretch of up to 1.8 times its length, cross-linked fibrin films can recover their original form [60]. Uncross-linked fibrin clots cannot achieve this [61]. Figure 3 illustrates why transverse interstrand positioning of cross-linked γ -chains is critically important for conferring this viscoelastic property.

Regulation of factor XIII activity in plasma

Plasma FXIII circulates as an A₂B₂-tetramer that is bound by its B subunits to γ -chain containing fibrinogen molecules ('fibrinogen 2') [62]. Fibrinogen 2 thus serves as the carrier protein for circulating factor XIII, and as discussed below, also serves to regulate its activity. Although plasma factor XIII provides a potent source of cross-linking activity in plasma, its activity usually is effectively suppressed. In contrast to thrombin-activated XIIIa, FXIII is virtually inactive in a non-physiological cadaverine-casein system [42]. Nevertheless, 'native' FXIII displays constitutive enzymatic activity at physiological calcium levels against preferred substrates such as fibrinogen or fibrin [42]; factor XIII-mediated cross-linking rates, especially of fibrin, can approach those observed with XIIIa. The 'intrinsic' XIII cross-linking activity includes α_2 -antiplasmin (α_2 -PI), which is found covalently cross-linked

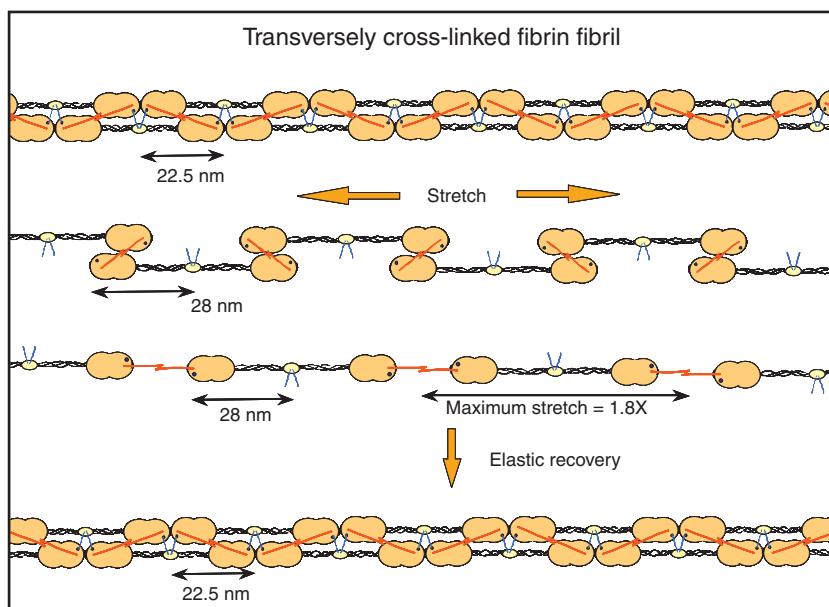


Fig. 3. Schematic diagram of a transversely cross-linked fibrin fibril that undergoes deformation because of the stress of stretching, and its elastic recovery after relaxing the stress. When a double-stranded fibril is maximally stretched 1.8 times [60], it becomes single-stranded, and fibril constituents remain ‘connected’ through the covalently cross-linked γ -chains. After stress relaxation, the fibrin film recovers its original form. Only cross-linked γ -chains that are positioned transversely between fibril strands, as shown, can account for this viscoelastic behavior (adapted from Mosesson [56]).

to plasma fibrinogen [63]. We recently extended this observation by demonstrating that native FXIII mediates cross-linking between α_2 -PI and fibrinogen (K. R. Siebenlist, M. W. Mosesson and P. A. McKee, unpublished experiments).

Under normal circumstances only small amounts of cross-linked fibrin(ogen) are detectable in plasma, thus raising the question as to how factor XIII activity is regulated in circulation. First, heterodimeric γ -chain containing fibrinogen 2 becomes cross-linked several times more slowly than homodimeric fibrinogen 1 molecules ($\gamma\text{A}/\gamma\text{A}$; Fig. 4), suggesting that fibrinogen 2 itself provides the means for suppressing factor XIII cross-linking activity. Secondly, excess B subunits

in plasma may provide another suppressive element, since B subunits prevent thrombin-independent activation of tissue factor XIII (A_2) [64], and their presence in the A_2B_2 -tetramer causes a lag in the onset of fibrinogen cross-linking by plasma XIII [42]. Other as yet undiscovered XIII regulatory mechanisms might exist.

Antithrombin I

Thrombin binding to its substrate, fibrinogen, is mediated through a fibrinogen recognition site in thrombin, termed ‘exosite 1’ [65,66]. Thrombin–fibrinogen substrate binding results in cleavage and release of FPA and eventually FPB, but concomitantly the resulting fibrin exhibits residual non-substrate thrombin-binding potential. The thrombin-binding activity associated with fibrin formation in clotting plasma was first termed ‘antithrombin I’ (‘AT-I’) by Seegers [67–69], and we still prefer this terminology. AT-I is now defined by two classes of non-substrate thrombin-binding sites in fibrin [70,71], one of low affinity in the E domain (approximately two sites per molecule), and the second of higher affinity in D domains of fibrin(ogen) molecules containing a γ -chain [71]. γ -Chains comprise approximately 8% of the total γ -chain population in plasma fibrinogen [7,9].

Low affinity thrombin-binding activity in fibrin reflects residual aspects of thrombin exosite 1 binding at the substrate site, and N-terminal residues in α -, β -, and γ -chains contribute to its formation [17,71–73], as recently detailed by Pechik *et al.* [74] based on crystal structures. The so-called ‘high affinity’ thrombin-binding site in fibrin(ogen) is situated in the C-terminal aspect of γ -chains between residues 414 and 427,

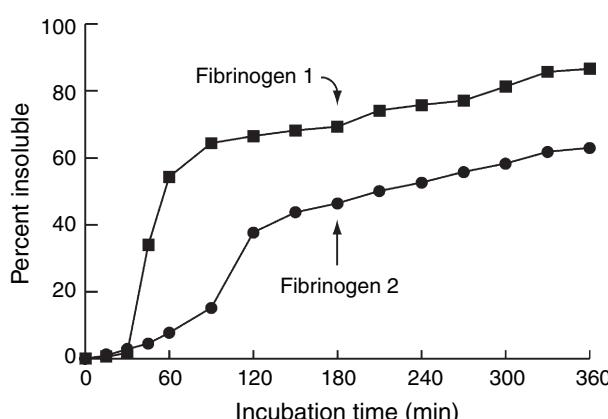


Fig. 4. The rates of fibrinogens 1 and 2 cross-linking mediated by plasma factor XIII at equivalent concentrations. The introduction of cross-links renders fibrinogen insoluble in acetic acid, and measuring this property provides a quantitative measure of the cross-linking rate (reproduced from Siebenlist *et al.* [42] with permission).

and sulfated tyrosine residues at $\gamma'418$ and $\gamma'422$ increase thrombin-binding potential [8]. Because thrombin 'exosite 2' binds fibrin at the γ' -site [75,76], thrombin binding to γ' chain-containing fibrin molecules is divalent, and this event increases the apparent association constant for high affinity thrombin binding [77].

Several studies of fibrin-bound thrombin have focused on the prothrombotic potential of thrombin bound to fibrin or to fibrin degradation products [78–83]. Currently, it is not as widely appreciated that thrombin binding to fibrin in clotting blood (i.e. AT-I) significantly suppresses thrombin generation. Although an AT-I deficiency or defect, *per se*, is generally not considered to be a thrombotic 'risk factor', this certainly appears to be the case, as described below.

An absence or deficiency of AT-I predisposes to thromboembolic disease, and this idea is based upon several observations and reports: (i) fibrin from certain dysfibrinogens, e.g. fibrinogen New York I [70] and fibrinogen Naples I [77,84,85], both of which exhibit reduced thrombin-binding capacity, are associated with severe venous or arterial thromboembolism. (ii) Thromboembolic disease, both venous and arterial, occurs in congenital afibrinogenemia or hypofibrinogenemia often in association with the therapeutic infusion of fibrinogen [86–95]. (iii) There is an increased level of thrombin generation in afibrinogenic plasma [95,96], and this can be normalized *in vitro* by the addition of fibrinogen. The demonstration that adding fibrinogen 2 ($\gamma A/\gamma'$) was more effective in normalizing thrombin generation than was fibrinogen 1 ($\gamma A/\gamma A$) [96], underscores the important role played by fibrinogen in this process, and more particularly γ' -chain containing fibrinogen. (iv) When measured in afibrinogenic plasma, there are increased levels of prothrombin activation fragment F_{1+2} [94,97] and thrombin-antithrombin III (TAT) complexes [95,97], and these markers of increased thrombin generation are normalized by infusion of fibrinogen, further suggesting that a hypercoagulable state exists in this condition. (v) The predisposition to occlusive arterial thromboembolism in an afibrinogenic subject who developed occlusive peripheral arterial thrombosis even in the absence of a fibrinogen infusion [95] is analogous to studies of ferric chloride-injured afibrinogenic mice, which showed abundant intravascular thrombi forming at the injury site that subsequently embolized downstream [98].

Fibrinogen and fibrinolysis

The exposure of tPA- and plasminogen-binding sites in fibrin Tissue-type plasminogen activator (tPA) is synthesized by vascular endothelial cells [99] and circulates in blood [100]. The tPA-mediated plasminogen activation is accelerated in the presence of fibrin, but there is little or no stimulatory effect in the presence of fibrinogen [101,102]. Nevertheless, there is a high affinity plasminogen-binding site in fibrinogen [103] that is located in the distal portion of each αC -domain; also there is a tPA-binding site in this same region [104]. Studies of fibrinogen Dusart ($A\alpha R554C$ -albumin), which showed defective

plasminogen binding, and fibrinogen Marburg, which lacks substantial portions of the αC -domain, showed impaired fibrin-stimulated plasminogen activation by tPA [105–108]. These results suggest that plasminogen binding in the αC -domain may regulate fibrinolysis by making bound plasminogen readily available for ternary complex formation in a fibrin system.

tPA-Stimulated plasminogen activation is strongly promoted by fibrin polymers, and also by cross-linked fibrinogen polymers [109]. Plasminogen activation occurs through tPA binding to fibrin followed by the addition of plasminogen to form a ternary complex [101]. Subsequent proteolytic cleavage of fibrin by plasmin creates additional lysine-binding sites [110,111], thereby enhancing fibrinolysis by increasing plasminogen accumulation. Two sites in fibrin are involved in enhanced plasminogen activation by tPA, $A\alpha 148–160$ and $\gamma 312–324$ [112–114]. Both sites are cryptic in fibrinogen but become exposed during fibrin assembly, primarily as a consequence of intermolecular D:E interactions that induce conformational changes in the D region and result in exposure of tPA- and plasminogen-binding sites. The exposure is reversed after the complex dissociates [115]. $A\alpha 148–160$ binds tPA or plasminogen with similar affinity [116–118]. However, plasminogen is preferentially bound at this site *in vivo* because the circulating plasminogen concentration is much higher than that of tPA [115,119]. The $\gamma 312–324$ sequence interacts exclusively with tPA [120,121].

Proteins that bind to fibrin(ogen) and affect fibrinolysis The plasmin inhibitor, α_2 -PI can be cross-linked to fibrin α -chains at $A\alpha 303$ [122,123], and its presence enhances resistance to fibrinolysis [124]. It was recognized many years ago that there was a potent antiplasmin activity associated with fibrinogen [125], but its identification as α_2 -PI remained uncertain until years later with the demonstration that α_2 -PI was covalently bound to plasma fibrinogen [63]. As discussed earlier, native FXIII is capable of cross-linking fibrinogen [42] and it is also capable of incorporating α_2 -PI into fibrinogen. In addition, 'PAI-2', an inhibitor of plasmin generation by urokinase or tPA, can be cross-linked to fibrin at several sites in the αC -domain that are remote from the $A\alpha 303$ site for α_2 -PI [126,127], thus amplifying the resistance of fibrin to lysis.

Lipoprotein(a) [LP(a)] is an atherogenic LP complex formed from apoLP(a), i.e. disulfide bound to the apoLP B-100 moiety of LDL. LP(a) binds to plasmin-degraded fibrin and fibrinogen [128], to the αC -domain by a lysine-independent mechanism [129], competes with plasminogen for binding sites in fibrin(ogen) [130,131], and becomes cross-linked to fibrinogen in the presence of FXIIIa [132]. Its presence on fibrin(ogen) has an inhibitory effect on fibrinolysis [130,131] and provides a mechanism for depositing LP(a) at places of fibrin deposition such as injured blood vessels or atherosclerotic lesions.

Histidine-rich glycoprotein Histidine-rich glycoprotein (HRGP) is a plasma and platelet protein that binds specifically to fibrinogen and fibrin [133]. A high proportion of HRGP

circulates as a complex bound to the lysine-binding site of plasminogen and this serves to reduce the effective plasminogen concentration, inhibit binding of plasminogen to fibrin(ogen), and retard fibrinolysis *in vitro* [134]. Although the physiologic relevance of the HRGP effect has been questioned [135], the fact remains that both high plasma levels of HRGP [136,137] or HRGP deficiencies [138–140], are associated with thrombophilia.

Molecular and cellular interactions of fibrin(ogen)

Heparin and endothelial cell binding In addition to its role in mediating fibrin assembly, the fibrin β 15–42 sequence binds heparin [141–143], thereby participating in cell–matrix interactions. The β 15–42 sequence mediates platelet spreading [144], fibroblast proliferation [145], endothelial cell spreading, proliferation and capillary tube formation [142,145–147], and release of von Willebrand factor [148,149]. More recently a dimeric heparin-binding fragment containing the β 15–57 sequence ' $(\beta$ 15–66)₂' was shown to mimic the high affinity heparin binding characteristics of fibrin or the fibrin E fragment [143]. The presence of the fibrinogen B β 1–14 fibrinopeptide segment interferes with heparin binding.

Interaction between the fibrin β 15–42 sequence and the endothelial cell receptor, vascular endothelial (VE)-cadherin, stimulates capillary tube formation and promotes angiogenesis [147,150]. A dimeric β -chain peptide (β 15–66)₂ has a much higher affinity [151], thus indicating that the VE-cadherin site in fibrin involves two fibrin β -chain segments.

Integrin binding to platelets Many cellular interactions with fibrinogen and fibrin occur through integrin binding to arg-gly-asp (RGD) sequences at α 5 β 1 572–575 (RGDS) and possibly at α IIb β 3 95–98 (RGDF) [152–156]. Cross-linking of α C-domains promotes integrin-dependent cell adhesion and signaling [157]. In the case of platelets, RGD sites compete with the fibrinogen γ A 400–411 sequence [158] (Fig. 1) for binding to platelet integrin α IIb β 3 [159–161]. The subject of fibrinogen–platelet interactions has recently been reviewed [162].

Fibrin(ogen) and the leukocyte integrin receptor α M β 2

The leukocyte integrin, α M β 2 (Mac-1), is a high affinity receptor for fibrin(ogen) on stimulated monocytes and neutrophils [163,164] that is important for fibrin(ogen)–leukocyte interactions that contribute to the inflammatory response, as lucidly discussed by Flick *et al.* [165]. The Mac-1-binding site within the fibrinogen D domain contains two peptide sequences, γ 190–202 and γ 377–395, now designated P1 and P2, which form adjacent antiparallel β -strands [166]. P1 is an integral part of the γ -module whereas P2 is inserted into the γ -module and forms an antiparallel β -strand with P1, as demonstrated in the X-ray structures of this region [44,167] (Fig. 5). In further experiments concerned with expression of the Mac-1-binding site in fibrin(ogen), Ugarova *et al.* [168] found that deletion of the minimal recognition sequence, γ 390–395, was not sufficient to ablate Mac-1 binding, because mutants were still effective in supporting adhesion of Mac-1-expressing cells. Yakovlev *et al.* [169] extended these observations by identifying a second sequence in the P1 segment between γ 228 and γ 253 that contributed to the binding activity.

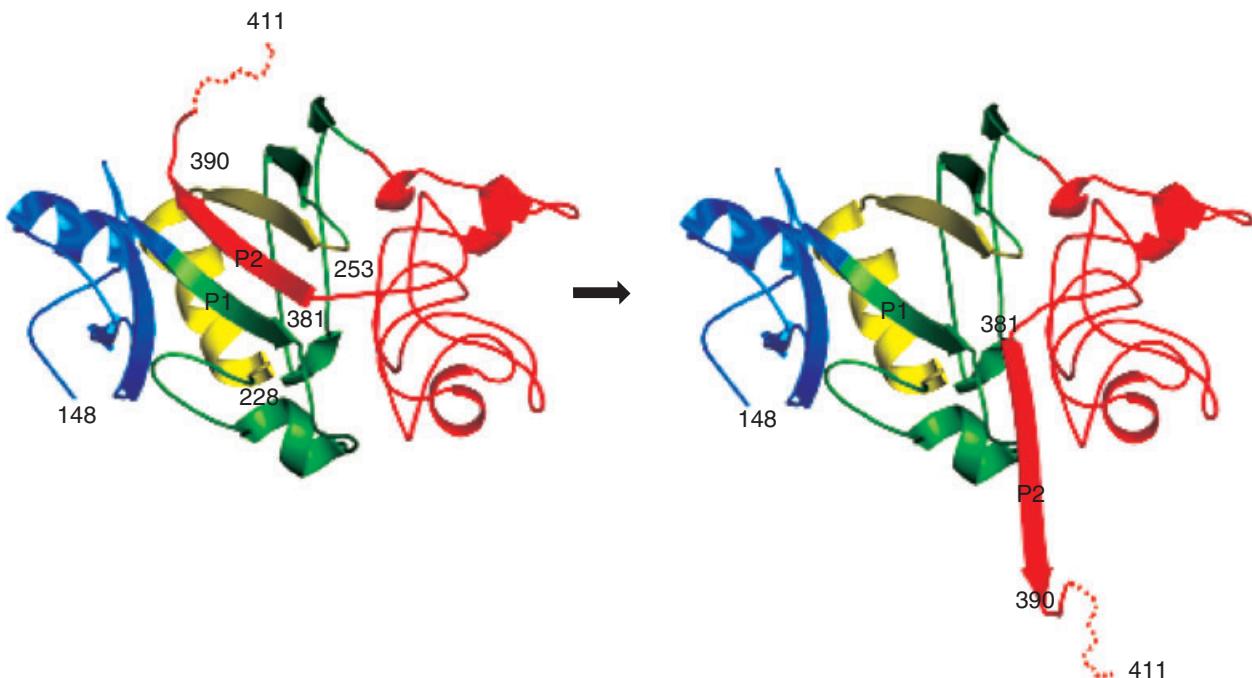


Fig. 5. Schematic ribbon diagram of the known γ -module crystal structure (residues γ 148–411; left), and the hypothetical structure resulting from 'pull out' of the γ 381–390 β -strand insert (P2; right; adapted from Yakovlev *et al.* [169]).

Exposure of the $\alpha_M\beta_2$ -binding site and the ‘pull out’ hypothesis Fibrin or immobilized fibrinogen bind to Mac-1 with great avidity, whereas soluble fibrinogen is a relatively poor ligand [170,171]. This indicates that the sites for Mac-1 are cryptic in fibrinogen but become exposed when fibrinogen is immobilized on plastic or converted to fibrin, clearly suggesting that significant conformational changes occur in this region of the D domain. Other evidence indicating conformational changes in this region involves the exposure of a receptor-induced binding site (RIBS-1) that has been localized to γ 373–385 [172,173]. The γ 373–385 sequence is recognized only after the platelet fibrinogen receptor, $\alpha_{IIb}\beta_3$, binds to fibrinogen.

Based upon X-ray structures of the D domain and the D domain γ -module [44,167] it has been assumed that the observed folding of β -strands in this region is immutable [174]. This has provided the rationale for dismissing the possibility of transverse cross-linking, in that the folding revealed in X-ray structures would not permit the emerging C-terminal segment of the γ -chain to extend far enough from the D domain to become engaged in ‘transverse’ cross-linking. This reasoning thus abnegates the possibility of transverse cross-linking without considering the large body of biochemical and biophysical evidence that favors it [56,57].

The question of β -strand folding has been examined by Yakovlev *et al.* [175] who showed that the P2 segment containing the γ 381–390 sequence could be displaced without disrupting the compact structure of the γ -module. This finding led to a so-called ‘pull out’ hypothesis (Fig. 5), which suggested that unfolding of the P2 β -strand insert could enable γ -chain cross-linking sites to extend sufficiently to become aligned for transverse cross-linking to take place, thereby allaying the conceptual objections raised above. Evidence recited earlier concerning exposure of Mac-1-binding sites as well as the RIBS-1 epitope, bolsters the pull out hypothesis because unfolding of P2 could readily account for their exposure.

Proteins, growth factors, and cytokines that bind to fibrin(ogen)

In addition to the many protein/molecular–fibrin(ogen) interactions covered in this article, some other examples of proteins that bind to fibrin(ogen) and affect its biological behaviors are listed below. Plasma fibronectin binds to the A α -chain of fibrinogen in its C-terminal region, since fibrinogen molecules lacking this part of the molecule do not interact with fibronectin [176]. Covalent cross-linking of fibronectin to fibrin [122,177] takes place mainly between Gln 3 of fibronectin [178] and the C-terminal region of the A α -chain of fibrin [179]. Such interactions would be advantageous for incorporating fibrin(ogen) molecules into the extracellular matrix.

Fibroblast growth factor-2 (FGF-2, bFGF) and vascular endothelial growth factor (VEGF) bind to fibrinogen and are able to potentiate endothelial cell proliferation when bound [180–182]. The cytokine, interleukin (IL)-1, is a participant in the inflammatory response. IL-1 β but not IL-1 α bound to fibrin(ogen), displaced bound FGF-2, and displayed enhanced

stimulatory activity of endothelial cells in the bound form [183]. These additional examples of fibrin(ogen) binding interactions are not intended to be exhaustive in scope, but rather they further illustrate how fibrin(ogen) participates in regulating its own physiological destiny.

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