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Abstract

Fibrinogen and fibrin play important, overlapping roles in blood clotting, fibrinolysis, cellular and matrix interactions, inflammation, wound healing, and neoplasia. These events are regulated to a large extent by fibrin formation itself and by complementary interactions between specific binding sites on fibrin(ogen) and extrinsic molecules including proenzymes, clotting factors, enzyme inhibitors, and cell receptors. Fibrinogen is comprised of two sets of three polypeptide chains termed Aα, Bβ, and γ, that are joined by disulfide bridging within the N-terminal E domain. The molecules are elongated 45-nm structures consisting of two outer D domains, each

connected to a central E domain by a coiled-coil segment. These domains contain constitutive binding sites that participate in fibrinogen conversion to fibrin, fibrin assembly, crosslinking, and platelet interactions (e.g., thrombin substrate, Da, Db, γXL, D:D, αC, γA chain platelet receptor) as well as sites that are available after fibrinopeptide cleavage (e.g., E domain low affinity non-substrate thrombin binding site); or that become exposed as a consequence of the polymerization process (e.g., tPA-dependent plasminogen activation). A constitutive plasma factor XIII binding site and a high affinity non-substrate thrombin binding site are located on variant γ′ chains that comprise a minor proportion of the γ chain population. Initiation of fibrin assembly by thrombin-mediated cleavage of fibrinopeptide A from Aα chains exposes two E_A polymerization sites, and subsequent fibrinopeptide B cleavage exposes two E_B polymerization sites that can also interact with platelets, fibroblasts, and endothelial cells. Fibrin generation leads to end-to-middle intermolecular Da to E_A associations, resulting in linear double-stranded fibrils and equilaterally branched *trimolecular* fibril junctions. Side-to-side fibril convergence results in *bilateral* network branches and multistranded thick fiber cables. Concomitantly, factor XIII or thrombin-activated factor XIIIa introduce intermolecular covalent ε-(γ glutamyl)lysine bonds into these polymers, first creating γ dimers between properly aligned C-terminal γXL sites, which are positioned *transversely* between the two strands of each fibrin fibril. Later, crosslinks form mainly between complementary sites on γ chains (forming γ-polymers), and even more slowly among γ dimers to create higher order crosslinked γ trimers and tetramers, to complete the mature network structure.

INTRODUCTION

Fibrinogen and fibrin play important, overlapping roles in blood clotting, fibrinolysis, cellular and matrix interactions, inflammation, wound healing, and neoplasia. These events are regulated to a large extent by the fibrin formation process and by interactions between specific sites on fibrinogen or fibrin and extrinsic molecules such as proenzymes, clotting factors, growth factors, enzymes inhibitors, and cell receptors. Fibrinogen molecules are elongated 45-nm structures consisting of two outer D domains, each connected by a coiled-coil segment to a central E domain (see **Figure 1**). They are comprised of two sets of three polypeptide chains termed Aα, Bβ, and γ,[1] which are joined together within its N-terminal E domain by five symmetrical disulfide bridges, one pair at position Aα 28, two pairs between Aα36 and Bβ65, and a third set between the γ8 and γ9 positions.[2-4] Because these γ chain bridges are reciprocal (i.e., γ8 to γ9),[5] they orient the chains in an antiparallel manner, and this may contribute to the twofold axis of symmetry perpendicular to the long axis that fibrinogen displays.[6-8] Other non-symmetrical interchain disulfide bridges in this region form a so-called *disulfide ring*.[2, 4] The Aα chain consists of 610, the Bβ chain 461, and the major form of γ chain, γA, 411 residues.[1] A minor γ chain variant termed γ′, arises through alternative processing of the primary mRNA transcript,[9] and amounts to about 8% of the total γ chain population.[10] γ′ chains consist of 427 residues and differ from the platelet-binding γA chains in that the four C-terminal γA residues, AGDV, are replaced by an anionic 20-amino acid sequence that includes two sulfated trosines.[11, 12] Fibrinogen γ′ chains bind plasma factor XIII B subunits and serve thereby as a carrier protein for the catalytic A subunits,[13] but they do not bind to the platelet fibrinogen receptor, $\alpha_{IIb}\beta_3$.[14] The C-terminal γA chain sequence, γ400–411, however, plays a critical role in mediating platelet aggregation via the platelet fibrinogen receptor.[15-17]

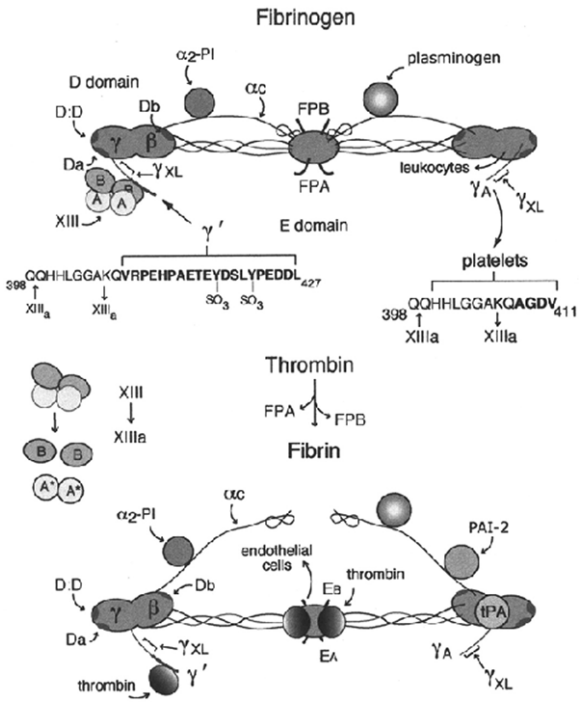

Figure 1 Schematic diagram of fibrinogen and fibrin showing the major structural domains, the association sites that participate in fibrin polymerization and crosslinking, and other molecular and cellular binding interactions.

FIBRINOGEN CONVERSION TO FIBRIN AND FIBRIN ASSEMBLY

The N-terminal region of each Aα chain contains the FPA sequence, cleavage of which at Aα16R–17G by thrombin initiates fibrin assembly[18-20] by exposing a polymerization site, E_A. One portion of the E_A site is located at the N-terminus of the fibrin α chain comprising residues 17 to 20 (GPRV);[21] another portion is located in the fibrin β chain between residues 15 and 42.[22-25]

Each E_A site subsequently combines with a constitutive complementary binding pocket (Da) in the D domain of neighboring molecules that is located between γ337 and γ379.[26-28] These initial E_A:Da associations result in formation of double-stranded twisting fibrils in which fibrin molecules become aligned in an end-to-middle staggered overlapping domain arrangement (see **Figure 2**).[29-31] Fibrils undergo lateral associations and form branches that result in a complex fiber network.[32, 33] Two types of branching account for fibrin network structures,[34] and they both play important roles in defining clot network structure. The first type occurs when double-stranded fibrils converge side-to-side to form the most widely appreciated fibril junction, a *tetramolecular* or *bilateral* branch point. More extensive lateral fibril associations result in large fiber bundles consisting of multiple fibrils and more condensed bilateral branch structures. This type of structure confers strength and rigidity to the network fibers.

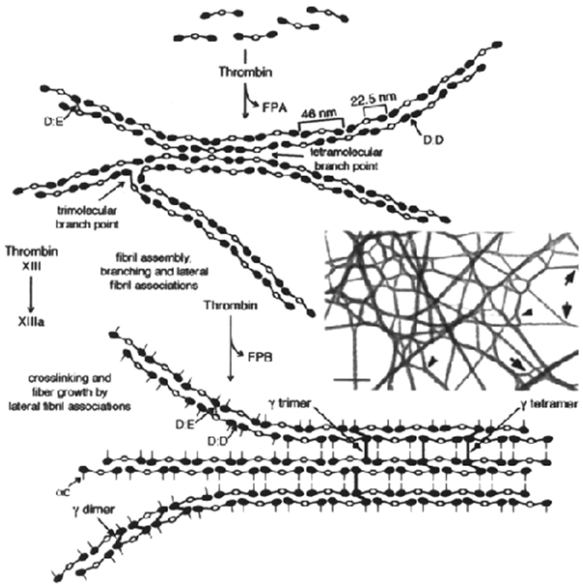

Figure 2 Schematic diagram of fibrin assembly and crosslinking. Assembly of fibrin begins with non-covalent interactions (D:E) between the EA and Da sites (*dotted lines*) to form end-to-middle staggered overlapping double-stranded fibrils (*upper*). Fibrils also branch and undergo lateral associations to form wider fibrils and fibers. [**Inset**: critical point dried thin fibril matrix containing equilateral (*arrows*) and bilateral (*arrowheads*) branch junctions; bar, 100 nm]. After cleavage of FPB (*lower*), αC domains become available for self-association with other αC domains, thereby promoting lateral fibril associations and fiber assembly. Factor XIII or XIIIa introduces ε-(γ-glutamyl) lysine isopeptide bonds between C-terminal γ_{XL} sites (*thick lines* between D domains) to rapidly form γ dimers. γ Trimers and γ tetramers form more slowly by interfibril γ chain crosslinking, and increase the resistance of the clot to fibrinolysis.

The second type of branch junction, termed *trimolecular* or *equilateral*, forms by the coalescence of three fibrin molecules that connect three double-stranded fibrils of equal widths (**Fig. 2**). Equilateral branches probably form with greater frequency when fibrinopeptide cleavage is relatively slow. Under these conditions, especially during the early phases of fibrin assembly, relatively large amounts of fibrin intermediates lacking a single FPA (des-A fibrin)[35, 36] are formed. The availability and persistence of intermediate fibrin units very likely promotes higher degrees of equilateral branching as partially converted des-A fibrin intermediates search for complementary partners with only partial success. Blombäck *et al.*[37] have shown that the architecture of fibrin networks formed at low levels of thrombin is more branched and therefore tighter (i.e., less porous) than networks formed at high levels of thrombin. The type of network architecture that characterizes the tight fibrin network is entirely consistent with the higher degree of equilateral fibril branching that occurs at low thrombin levels.[38] Such branches enhance clot elasticity.

There are two constitutive self-association sites, γ_{XL} and D:D, that participate in the fibrin or fibrinogen assembly process.[39, 40] The γ_{XL} site overlaps the crosslinking site in the C-terminal region of each γ chain. Intermolecular association between two γ_{XL} sites promotes alignment of crosslinking sites for subsequent factor XIII- or XIIIa-mediated transglutamination. The D:D sites are situated at the outer portion of each fibrin(ogen) D domain between residues 275 and 300 of the γ module.[41] They are necessary for proper end-to-end alignment of fibrinogen or fibrin molecules in assembling polymer structures, as exemplified by investigation of a dysfibrinogenemia, Tokyo II (γR275C),[40] which had defective D:D contacts. Crosslinked Tokyo II fibrinogen formed disorganized fibril structures despite the fact that γ chain crosslinking at the γ_{XL} site had proceeded at a normal rate. Tokyo II fibrin networks were characterized by increased fiber widths, tapering fibers, and an

increased level of fiber branching, which evidently resulted from slower fibrin assembly and inaccurate end-to-end positioning of assembling fibrin monomers.

MOLECULAR AND CELLULAR INTERACTIONS FOLLOWING FIBRINOPEPTIDE B CLEAVAGE

FPB (Bβ1–14) release occurs more slowly than the release of FPA[18-20] and exposes an independent polymerization site, E_B,[42] beginning with β15–18 (GHRP).[21] E_B is utilized through interactions with a constitutive complementary D_b site located in the C-terminal β chain segment of the D domain.[28, 42, 43] The E_B:D_b interaction is not absolutely required for lateral fibril and fiber associations, but it contributes to this process through cooperative interactions resulting from alignment of D domains in the assembling polymer.[42] Polymerization of des BB-fibrin results in the same type of fibril structure as occurs with des AA-fibrin,[38] but the clot strength is lower than that of des AA-fibrin.[42]

The αC domain originates in the D domain at residue 111 and terminates at its C-terminus, Aα610.[1] Fibrin clots formed from circulating fibrinogen molecules such as fibrinogen *catabolite* fractions I-6 to I-9, which lack C-terminal portions of the αC domain, display a prolonged thrombin time, reduced turbidity, and produce thinner fibers.[44-46] In fibrinogen, the αC domains tend to be non-covalently tethered to the E domain,[6, 47, 48] but dissociate from it following FPB cleavage.[47, 48] This event evidently makes αC domains available for interaction with other αC domains, thereby promoting lateral fibril associations and network assembly (**Fig. 2**).

In addition to its role in mediating fibrin assembly, the β15–42 sequence binds heparin[49, 50] and participates in cell-matrix interactions. β15–42 mediates platelet spreading,[51] fibroblast proliferation,[52] endothelial cell spreading, proliferation and capillary tube formation,[50, 52-54] and release of von Willebrand factor.[55, 56] Binding to endothelial cells is mediated by the endothelial cell receptor, VE-cadherin.[54]

CROSSLINKING OF FIBRINOGEN AND FIBRIN

The C-terminal region of each fibrinogen or fibrin γ chain contains a single crosslinking site at which factor XIII or XIIIa catalyzes the formation of γ dimers[39, 57-59] by incorporating intermolecular reciprocal ε-(γ-glutamyl)lysine bridges between a donor γ406 lysine of one chain and a glutamine acceptor at γ398/399 of another.[60-62] The same type of intermolecular cosslinking occurs more slowly among several amine donor and lysine acceptor sites in Aα or α chains,[32, 65] and small amounts of internally crosslinked α-γ chain heterodimers are found in plasma fibrinogen molecules.[66] In addition to γ dimers, higher order forms of crosslinked γ chains, namely γ trimers and γ tetramers, evolve slowly.[32, 59, 65, 67] Because there is only a single donor lysine residue at γ406,[60, 62, 63, 68] it is safe to assume that trimeric and tetrameric structures form through utilization or reutilization of that same residue.

THE ALIGNMENT OF γ CHAIN PAIRS IN CROSSLINKED FIBRINOGEN OR FIBRIN POLYMERS

Interactions between D and E domains in an assembling fibrin polymer facilitate the antiparallel intermolecular alignment of γ chain pairs at the γ_{XL} site, thereby accelerating the rate of XIIIa-mediated crosslinking.[58, 39] The orientation of these C-terminal γ chain pairs within an assembled and crosslinked fibrin or fibrinogen polymer has been at issue for some time. On the basis of evidence to be recited below, we believe that crosslinked γ chains become situated *transversely* between the D domains of opposing strands of a fibrin or fibrinogen fibril (see **Figure 3**). Despite the strength of this evidence, many others hold to the belief that these crosslinked regions are positioned across the distal ends of two linearly aligned molecules within a fibril strand in a so-called *DD-long* or end-to-end arrangement. It is valuable to summarize the evidence for these differing views,

beginning with the end-to-end argument. Soon after the report by Fowler *et al.*,[69] which involved the demonstration of XIIIa-crosslinked fibrinogen dimers disposed end-to-end, it became almost axiomatic that crosslinked γ chains in assembled fibrin fibers were oriented in the same way. The DD-long model later drew support from observations on crosslinked fibrin degradation products that had been unfolded in acetic acid[70] and from the appearance of end-to-end crosslinked fibrinogen molecules that had been dissociated in acetic acid or that had been assembled on a fibrin fragment E template.[71] The conclusions drawn in both studies were ambiguous because they were based upon the appearance of acetic acid dissociated polymer structures that are known to become unfolded under such conditions.[72] In addition, side-to-side fibril associations, as well as end-to-end associations occurred in the fibrin E-fibrinogen study,[71] making it possible for either end-to-end or transverse crosslinking to occur. At most, these studies show that end-to-end positioning of crosslinked γ chains occurs under circumstances that are not closely related to those in assembled fibrin.

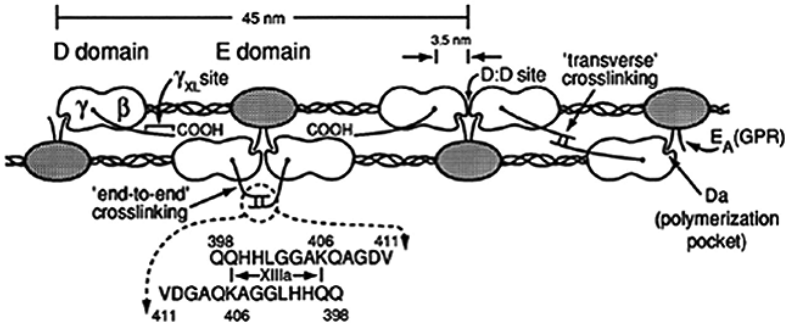

Figure 3 Schematic diagram of assembled fibrin molecules illustrating the intermolecular arrangements in a fibril. The positioning of the γ_{XL} sites in transverse or end-to-end orientations is shown, as is the C-terminal sequence of the critical residues of the γ_A chain crosslinking site. The emergence of the C-terminal portion of the γ chain from the middle of the γ module is consistent with recent crystallographic reports.[41, 73] Reproduced from Mosesson *et al.*[78]

The idea of a DD-long arrangement has also gained inferential support from evaluation of D domain crystal structures,[41, 73] even though in the studies cited the γ chain region involved in crosslinking had not been visualized. Nevertheless, from the observed folding of structures comprising the D domain crystals and from calculation of the distances that each γ chain would presumably be required to traverse in order to become engaged with another γ chain, these groups concluded that transverse crosslinking could not occur in fibrin. These calculated discrepancies do not amount to a cohesive argument against the transverse crosslinking arrangement since they do not directly contradict the experimental evidence that provides proof for such structures in fibrin (*vide infra*). They should serve instead as a stimulus to reconcile potential differences between structures found in D domain crystals and those that enable transverse crosslinking to take place in fibrin. In this connection, Medved *et al*.[74] recently reported that the middle strand (i.e., γ381 to γ390) of a five-stranded β sheet structure in the γ module of D domain crystals, immediately preceding the sequence containing the γ_{XL} site, can be displaced without disrupting the compact structure of the γ module. This suggests that the γ_{XL} region in the fibrin polymer can be *pulled out* and thus become more extended, a behavior that might explain how transverse crosslinking takes place.

Several investigations relating to fibrin crosslinking, beginning with one by Selmayr *et al.*,[75] point directly to transverse positioning of γ chains in crosslinked fibrin fibrils. These investigators found that in the presence of XIIIa, fibrinogen molecules formed a γ chain-crosslinked complex with immobilized fibrin, an arrangement that was interpreted as having arisen through transverse γ chain orientation. In later studies,[76] this group showed that crosslinked double-stranded fibrin fibrils were not depolymerized by 3 M urea, a solvent that can otherwise disrupt D:E interactions. The observation reinforced the conclusion that crosslinked γ chains were positioned

transversely in fibrin networks. In follow up studies triggered by the Selmayr reports,**a** electron microscopic analyses of crosslinked fibrinogen polymers demonstrated double stranded fibrils that had formed via crosslinked γ chains traversing the fibril strands[39] (see **Figure 4**). More focused evidence for transverse crosslinking came from a study demonstrating that mixtures of fibrin plus plasmic fragment D produced XIIIa-crosslinked D:fibrin:D complexes that must have come about through transverse γ chain positioning.[72] Moreover, EM images of D:fibrin:D complexes in that same study showed folding of the crosslinked molecular complex into transverse positions that could readily be converted to end-to-end positioning in the presence of dilute acetic acid, a solvent that disrupts non-covalent intermolecular fibrin associations. More recently, high resolution electron microscopy of gold-cadaverine-labeled γ chains in fibrinogen molecules or fibrin fibrils demonstrated that C-terminal γ chain regions are most often oriented toward the center of the molecule, suitably aligned for transverse crosslinking.[78] The most recent and perhaps most convincing evidence for transverse γ chain crosslinking, was obtained from analyses of the type and amount of radioactive γ dimers produced in crosslinking mixtures of fibrinogen 1 (γA, γA) and ^{125}I-labeled fibrin 2 (γA, γ′) (see **Figure 5**) or the converse, fibrin 1 and ^{125}I-fibrinogen 2 (not shown).[77] In the example shown, had DD-long crosslinking occurred, radioactive γ dimers would not have formed, whereas transverse crosslinking would have resulted in a 1:1 mixture of radioactive γA-γA and γA-γ′ dimers. In fact, a mixture of radioactive γ dimers in a 1:1 ratio was found as predicted for transverse crosslinking. In summary, although the possibility remains that some degree of end-to-end crosslinking may occur in fibrin, in sifting through all available information, we can find no durable evidence for this type of crosslinking in assembled fibrin networks. Instead, the evidence points to transverse crosslinking as the predominant, if not the only, crosslinking arrangement in fibrin.

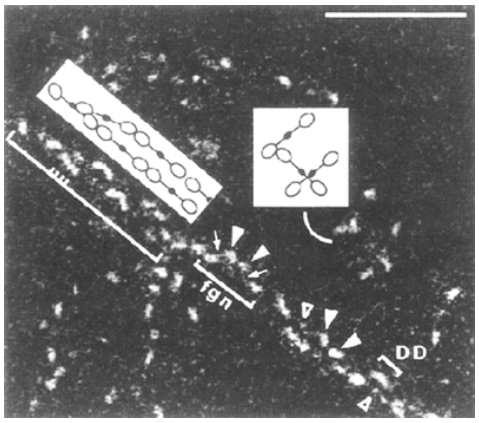

Figure 4 Scanning transmission electron microscope (STEM) image obtained from a sample of a XIIIa-fibrinogen crosslinking mixture. The micrograph shows a double-stranded γ chain-crosslinked fibrinogen fibril. The arrangement of the molecules comprising the fibril is drawn schematically. Filamentous structures connecting the fibril strands (*arrows*), probably represent transversely crosslinked γ chains. There are numerous non-crosslinked fibrinogen molecules and occasional oligomers in the field. *Bar*, 100 nm. Reproduced from Mosesson *et al*.[39]

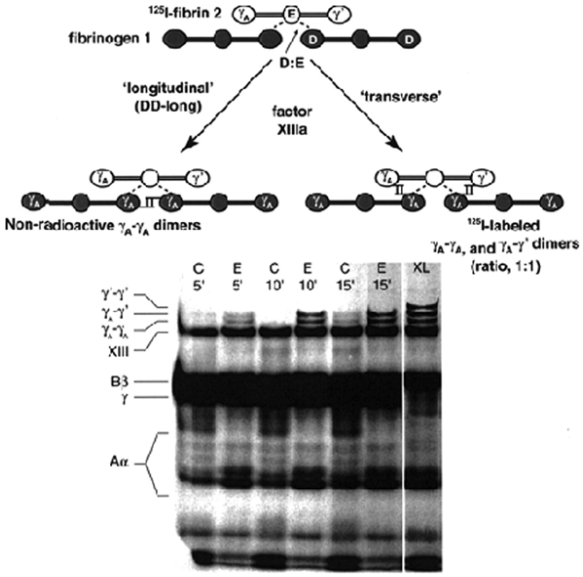

Figure 5 One of two protocols that were used to evaluate end-to-end versus transverse XIIIa-mediated crosslinking in a mixture of fibrinogen 1, ^{125}I-fibrin 2, and XIIIa (*upper*) and the results of autoradiography of a SDS-PAGE gel (*lower*). Modified from Siebenlist *et al.*[77]

FIBRINOGEN AND FIBRINOLYSIS

Tissue-type plasminogen activator (tPA)[79] circulates in blood and is synthesized by vascular endothelial cells.[80] tPA-mediated plasminogen activation is markedly accelerated in the presence of fibrin, whereas there is little effect in the presence of fibrinogen.[81, 82] The stimulatory event is promoted not only by fibrin polymers, but also by XIIIa-crosslinked fibrinogen fibrils.[83] Plasminogen activation occurs through tPA binding to fibrin followed by the addition of plasminogen to form a ternary complex.[81] Two sites in fibrin are involved in enhancement of plasminogen activation by tPA, Aα148–160 and γ312–324.[84, 85] These sites are cryptic in fibrinogen but become exposed as a consequence of non-covalent D:E interactions in assembling fibrin fibrils. These associations induce conformational changes in the D region that result in exposure of tPA- and plasminogen binding sites, and the exposure is reversed after the complex dissociates.[86] There is a single high affinity plasminogen binding site in fibrinogen or fibrin,[87] and it seems to be located in the distal portion of the αC domain.[88] Proteolytic cleavage by plasmin creates numerous additional lysine binding sites,[89, 90] thereby enhancing fibrinolysis by increasing the accumulation of plasminogen.

Other plasma proteins, notably α_2-antiplasmin, are crosslinked to fibrin α chains[91] at Aα303.[92] Although it has been recognized for many years that there is a potent antiplasmin activity that is bound to fibrinogen,[93] the specific entity in fibrinogen accounting for that activity had not been identified. Recently, however, Siebenlist *et al.*, using an immunochemical technique, directly demonstrated the presence of covalently bound α_2-antiplasmin in normal fibrinogen as well as in a dysfibrinogenemic fibrinogen, Cedar Rapids (γR275C).[94] The transglutaminase activity responsible for the effect is probably plasma factor XIII, which has considerable constitutive fibrinogen crosslinking potential.[59] The presence of α_2-antiplasmin on fibrin(ogen) enhances resistance to fibrinolysis,[95] but some other protein bound to fibrin(ogen) might also contribute to this effect. That is, in addition to α_2-antiplasmin, PAI-2, an inhibitor of plasmin generation by urokinase or tPA, can also be crosslinked to fibrin at sites in the Aα chain that are remote from the Aα303 site for α_2-antiplasmin,[96, 97] thus amplifying fibrin resistance to lysis.

Lipoprotein(a) is a highly atherogenic lipoprotein complex formed from apolipoprotein(a) that is disulfide bound to the apolipoprotein B-100 moiety of LDL. LP(a) binds to plasmin-degraded fibrin and fibrinogen,[98] competes with plasminogen for binding sites in fibrin(ogen),[99, 100] and becomes crosslinked to fibrinogen in the presence of

XIIIa,[101] effectively increasing the local concentration of LP(a) within the clot. The presence of this lipoprotein complex on fibrin(ogen) has an important negative effect on fibrinolysis.[99, 100] (Also see HRGP.)

THROMBIN BINDING TO FIBRIN AND FIBRINOGEN

Thrombin binding to its substrate fibrinogen is mediated through an anion-binding fibrinogen recognition exosite in thrombin.[102, 103] Binding at the substrate recognition sites in the E domain leads to proteolytic cleavage and release of FPA and FPB. In addition to fibrinogen-substrate binding, fibrin also has a significant thrombin binding potential that was highlighted more than 55 years ago by Walter Seegers, and which he termed antithrombin I.[104, 105] Little more has been written on this topic from the standpoint of its relevance to thrombin metabolism and the pathophysiology of thrombophilia, but we do know that dysfibrinogenemic subjects with defective thrombin binding to fibrin have strikingly thrombophilic phenotypes.[106]

Antithrombin I activity is defined by two classes of non-substrate thrombin binding sites in fibrin,[107, 108] one of low affinity in the E domain, and the other of high affinity in D domains of fibrin(ogen) molecules containing a γ chain variant termed γ′ (γ′1–427L).[108] γ′ chains contain a unique 20 residue highly anionic, tyrosine-sulfated C-terminal sequence (**Fig. 1**),[11, 12] and differ from γA chains, which instead contain a C-terminal platelet-binding sequence (γA400–411V).[109] Low affinity thrombin binding evidently reflects residual aspects of fibrinogen substrate binding, and both N-terminal portions of α chains (α27–50) and β chains (β15–42) contribute to their formation.[24, 108, 110, 111] The high affinity thrombin binding site in fibrin(ogen) is situated exclusively in the C-terminal aspect of γ′ chains (γ1–427L) between residues 414 and 427. Sulfated tyrosine residues at γ′418 and γ′422 significantly increase the thrombin binding affinity.[12]

There is good reason to believe that antithrombin I activity plays an important role in modulating thrombin activity. Dysfibrinogenemic subjects manifesting defective non-substrate thrombin binding develop severe thromboembolic disease,[106] most notably fibrinogens New York I (des Bβ9–72)[22, 112] and Naples I (Bβ A68T).[113-115] Fibrinogen New York I was associated with recurrent deep venous thrombosis and fatal pulmonary embolism.[112] Two homozygous members of the Naples I kindred experienced occlusive arterial strokes at an early age, whereas a third homozygous family member suffered DVT following abdominal surgery.[116] In addition to the reports cited above, recent *in vitro* experiments indicate that thrombin generation in recalcified afibrinogenemic plasma[117, 118] and in reptilase-defibrinated plasma[118] is significantly higher than in normal fibrinogen-containing plasma. Repletion of afibrinogenemic plasma with fibrinogen restores thrombin generation to normal. Fibrinogen 2, which contains both high and low affinity thrombin-binding sites has a more profound normalizing effect than fibrinogen 1 (low affinity site only).[117] The connection between antithrombin I activity and thrombotic disease remains to be more fully explored.

INTEGRIN BINDING SITES

Fibrinogen contains two integrin binding sites at Aα95–98 (RGDF) and at Aα572–575 (RGDS).[1] Many cellular interactions with fibrinogen and fibrin occur through binding to one or both of these recognition sequences. In addition to the ability of RGD sites to bind to platelet $\alpha_{IIb}\beta_3$ and compete for binding with the γA400–411 sequence,[120, 121] $\alpha_V\beta_3$ integrins on endothelial cells,[122] melanoma cells,[123] and fibroblasts[124] also mediate RGD-dependent fibrinogen binding. There are other integrins on endothelial cells or fibroblasts that bind to fibrin(ogen) RGD sites.[125, 126]

The leukocyte integrin *CD 11b/CD18 ($\alpha_M\beta_2$, Mac-1)* is a high affinity receptor for fibrinogen on stimulated monocytes and neutrophils that has been implicated in the inflammatory response. $\alpha_M\beta_2$ has been localized within the fibrinogen D domain at a site corresponding to the confluence of γ190–202 and γ377–395, which form two antiparallel β strands[127] (**Fig. 1**). It is valuable to recall that the γ381 to γ390 segment may undergo significant conformational rearrangements.[74]

OTHER BINDING INTERACTIONS

There are numerous examples of proteins that interact with fibrinogen or fibrin. The listing below is not intended to be exhaustive, but coupled with binding interactions covered elsewhere in this article, it reinforces the notion that fibrin(ogen) helps to orchestrate its own physiological destiny. *Plasma fibronectin* binds to the Aα chain of fibrinogen in its C-terminal region, since fibrinogen molecules lacking this part of the molecule do not interact with fibronectin.[128] Crosslinking of fibronectin to fibrin is mediated by factor XIIIa[91, 129] between several potential lys residues in the fibrin α chain[130] and mainly gln-3 of fibronectin.[131] *Fibroblast growth factor-2* (FGF-2, bFGF) binds to fibrinogen[132] and is able to potentiate endothelial cell proliferation.[133] Furthermore, binding to fibrinogen protects it from proteolytic degradation.[134] Another growth factor, *insulin-like growth factor-1* (IGF-1), becomes bound to *insulin-like growth factor-binding protein-3* (IGFBP-3) which itself binds directly to fibrinogen or fibrin. IGF-1 participates in the wound healing process by stimulating stromal cell function and proliferation.[135]

A vitamin K-dependent clotting factor, *Xa*, has been shown to bind to fibrin and to fibrin/fibrinogen degradation products.[136] Xa bound specifically to a peptide corresponding to Aα 82D to 123K, a site that is located between the E and D domains, and quite distinct from the thrombin binding sites. *Tissue factor pathway inhibitor* binds to fibrin via a C-terminal region that is rich in Arg and Lys residues, but the location of the binding site in fibrin has not been determined.[137] *Gelsolin* is an actin-binding plasma protein that severs actin filaments, and that has been shown to bind to the fibrin clot.[138]

Histidine-Rich Glycoprotein is a plasma and platelet protein that binds specifically to fibrinogen and fibrin.[139] 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, and retard fibrinolysis *in vitro*.[140] The physiological relevance of this effect has been questioned.[141] Nevertheless, both high plasma levels of HRGP,[142, 143] or HRGP deficiencies,[144, 145] are associated with venous thromboembolism.

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Footnotes

[a] M.W.M became interested in this problem as a member of the Scientific Review Committee for Professor Gert Müller-Berghaus' research group in Giessen, Germany, during which he had the opportunity of reviewing and discussing Eberhard Selmayr's experiments. These interactions stimulated our later interests in this subject.

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