Effects of Actin Filaments on Fibrin Clot Structure and Lysis

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The muscle and cytoskeletal protein actin is released from cells as a consequence of cell death and interacts with components of the hemostatic and fibrinolytic systems, including platelets, plasmin, and fibrin. We report here that incorporation of actin filaments into fibrin clots changes their viscoelastic properties by increasing their shear modulus at low deforming stresses and by nearly eliminating their tendency to become more rigid with increasing deformation (ie, exhibit strain-hardening). The viscoelastic effects depended on the length of the actin filaments as shown by the effects of the plasma filament-severing protein, gelsolin. Binding of actin to fibrin clots also varied with actin filament length. The plasma actin-binding proteins gelsolin and vitamin D-binding protein reduced, but did not eliminate, the incorporation of actin in the clot. Fluorescence microscopy showed a direct association of rhodamine-labeled actin filaments with the fibrin network. Incubation of clots containing long actin filaments in solutions containing physiologic concentrations of gelsolin (2 μmol/L) released 60% of the actin trapped in the clot. Reduction of the actin content of a fibrin clot by incubation in a gelsolin-containing solution resulted in an increased rate of clot lysis. The ability of plasma gelsolin to shorten actin filaments may therefore be of physiologic and potentially of therapeutic importance insofar as gelsolin-mediated diffusion of actin from the clot may restore the clot’s rheologic properties and render it more sensitive to the lytic action of plasmin.

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Tissue Injury results in the activation of the coagulation cascade, leading to the local deposition of fibrin. During inflammatory events, molecules not normally found in plasma may enter the extracellular space, potentially affecting blood coagulation. Some of these molecules may arise from the action of cellular proteases upon normal plasma constituents (eg, fibrinogen), the release of granules of inflammatory cells, or the disruption of cellular plasma membranes. Extensive studies of the effects of fibrinogen degradation products upon fibrin clot formation have provided important information about the mechanisms whereby inflammatory events lead to alterations in the process of blood coagulation. Molecules that alter the fibrin gel formation and clot structure may play a role in determining the hemostatic effectiveness of fibrin clots, as well as their ability to mediate fibrin-dependent fibrinolysis.

Actin, the predominant protein of nucleated cells and blood platelets, has been found at micromolar concentrations in the plasma or serum of animals and humans experiencing a variety of types of tissue injury. In vitro, this globular protein assembles into rodlike filaments many microns in length; in vivo, it exists rapidly between monomeric and polymeric forms, a process regulated in the cytoplasm by a number of actin-binding proteins. When added to fibrinogen solutions, actin filaments may interfere with the process of fibrin clot formation by sterically hindering the diffusion of fibrin protofibrils into bundles.

Mammalian blood plasma contains micromolar concentrations of two proteins that bind to actin with high affinity, plasma gelsolin and the vitamin D-binding protein (DBP; also known as GC globulin). Plasma gelsolin, a secreted homolog of a cytoplasmic protein, nonproteolytically shortens actin filaments by disrupting the noncovalent bonds between subunits and forms 1:2 complexes with actin monomers. DBP, on the other hand, binds only to monomeric actin. Both plasma gelsolin and DBP serve to clear actin from the circulation. DBP has recently been shown to prevent microvascular thrombosis brought about by the injection of monomeric actin into experimental animals. Plasma gelsolin and DBP thus constitute a defense system designed to protect the host against the deleterious effects of actin released into the circulation.

Recent findings concerning the interaction of actin with fibrinolytic proteins indicate the need for learning more about actin’s effects upon fibrin gel structure and function. Actin is able to promote plasmin formation when added to solutions containing Glu-plasminogen and tissue plasminogen activator (t-PA). However, when actin-containing clots are placed in a lysis bath containing plasminogen and t-PA, clot lysis is inhibited. While experiments with purified proteins suggest that actin is a noncompetitive plasmin inhibitor, an effect of actin on fibrin clot structure may also account for some of its inhibitory effects upon clot lysis. Because the rheology of fibrin clots is also an important aspect of their physiologic functioning, the studies reported here were undertaken to delineate further the effects of actin upon fibrin clots and to determine whether plasma actin-binding proteins have a significant modifying effect on this interaction.

Materials and Methods

Materials. Fibrinogen and gelsolin were purified from human blood plasma, and actin from rabbit skeletal muscle, by previously described methods. Actin was labeled with pyrene-iodoacetamide or tetramethylrhodamine 5-(and-6)-idoacetamide (T-488; Molecular Probes, Eugene, OR) as described elsewhere. DBP was either prepared from human blood by affinity chromatography using actin-Sepharose or purchased from Calbiochem (San Diego, CA). These materials were generously donated by Dr P. A. Janmey (Division of Experimental Medicine, Brigham and Women’s Hospital; the Surgery Research Unit, Surgical Services, Massachusetts General Hospital; and the Department of Chemistry, Roskilde University, Roskilde, Denmark).

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Diego, CA). Both materials showed similar affinity for actin based on their ability to inhibit actin polymerization as measured by changes in pyrene-labeled actin fluorescence. Thrombin from human plasma (T-6759; Sigma, St Louis, MO) and plasmin (810655; Kabi Vitrum, Franklin, OH) were diluted to 100 U/mL and 10 U/mL, respectively, with water and frozen immediately. Aliquots were thawed, kept at 4°C, and used within 10 hours. Rhodamine-labeled phalloidin was obtained from Molecular Probes, fluorescein-labeled avidin from Enzo Diagnostics (New York, NY), and DNease I from Boehringer Mannheim (Indianapolis, IN).

Viscoelastic measurements. The viscoelastic properties of fibrin gels were determined by rheologic measurements using either a Rheometrics (Piscataway, NJ) instrument or a torsion pendulum. The principle behind these measurements is that when a force is applied in a direction parallel to the face of a sample (a shear force, or stress = force/area given in units of Pa = 1 dyne/cm²) a viscoelastic sample deforms to an extent (the strain, a unitless quantity) that depends on the magnitude of the stress and on the length of time that the stress is applied. Part of the energy of deformation is stored elastically in the material and part is dissipated by the slow viscous motion of the sample, leading to irreversible deformation after the stress is removed. These viscoelastic properties can be quantitatively described by the shear modulus: the ratio of stress to strain, which is itself a function of time and, for some materials and extents of deformation, also of the strain. Often, the storage and loss (viscous) shear moduli are measured by applying oscillating deformations and then the shear modulus, termed \( G' \) for the storage and \( G'' \) for the loss modulus, are calculated from the magnitude and phase shift between oscillating stresses and strains. The Rheometrics device applies an oscillating shear deformation to a sample confined between a cone and a plate, and it enables measurements of the elastic storage modulus, \( G' \), as a function of time, frequency, or deformation amplitude. The torsion pendulum can either measure the dynamic shear modulus from free oscillation or the shear compliance (ratio of strain to stress) from measurements of the deformation (strain) when a constant shear stress is applied to a disk-shaped sample held between the plates of the pendulum. The principles of operation of these two instruments are described elsewhere.24,25 In both instruments, the fibrin or fibrin/actin gel is formed between the plates of the rheometer by placing 400 to 800 p,L of a protein solution on the bottom plate immediately after addition of thrombin to initiate fibrin polymerization. The thrombin concentration was chosen to give a clotting time of a few minutes, which permitted time to position the sample in the rheometer before clotting occurred. Measurements were typically started 1 minute after the sample was placed between the plates, and the clotting time was approximately 3 minutes. Measurements of strain dependence were made on samples aged for 90 minutes, or at least 30 times the clotting time, to ensure that clot formation was nearly complete and unchanged during the course of these measurements. When actin or actin/gelsolin complexes were added to the fibrinogen solution, G-actin was first polymerized to F-actin in the presence or absence of gelsolin by incubation for 1 hour in buffers containing 2 mmol/L MgCl₂ and 150 mmol/L KCl.

Confocal microscopy. Fibrinogen (2 mg/mL) was mixed with 6 \( \mu \)mol/L rhodamine-actin in the presence or absence of gelsolin or DB (at molar ratios to actin of 1:4 or 2:1, respectively). Fibrin polymerization was initiated by the addition of 0.1 U/mL thrombin and 10 \( \mu \)L of each solution was placed on a microscope slide in a humidified chamber consisting of a hydrated filter paper in a covered Petri dish. Approximately 5 minutes after addition of thrombin, at a time near the clotting time, a cover slip was applied to the sample and the edges sealed with nail polish. In a control experiment, 10 \( \mu \)mol/L fluorescein-labeled avidin was mixed with fibrinogen and polymerization initiated by addition of thrombin. Beginning 1 minute after preparation of the slides, samples were examined using a Bio-Rad MRC 600 scanning laser confocal microscope attached to a Zeiss Axiosvert microscope (Bio-Rad, Cambridge, MA). A 100× (NA 1.3) Plan-neofluar (Zeiss, Thornwood, NY) objective was used for imaging, and the confocal aperture was set at the minimum opening. This helped to eliminate fluorescence above and below the plane of focus, thereby increasing resolution and enabling us to visualize individual fibrin strands. The same laser setting, gain, and frames accumulated were used for all samples. This allowed for more accurate comparison of fluorescence intensities.

Binding experiments. The amount of actin bound to fibrin was measured by the loss of solution fluorescence after incorporation of rhodamine-labeled actin into 2 mg/mL fibrin clots. Clots were formed in borosilicate glass culture tubes by the addition of 1.7 NIH U/mL thrombin to 2 mg/mL fibrinogen in T7 (100 mmol/L NaCl, 50 mmol/mL Tris-Cl, pH 7.4) with F-actin, polymerized in buffer B (20 mmol/mL Tris, 0.5 mmol/L ATP, 0.2 mmol/L DTT, 0.2 mmol/L CaCl₂, 150 mmol/L KCl, 2 mmol/L MgCl₂, pH 7.4). Fibrin gelation occurred within 10 minutes and the clots were incubated for 1 to 2 hours at 24°C. The insoluble clots, containing greater than 97% of the total fibrin, were removed by washing onto a glass pipette, and the fluorescence of the remaining solution was determined. Before and after clot formation, fluorescence was determined with a Perkin Elmer LS-5B Luminescence Spectrometer (Perkin-Elmer Cetus, Norwalk, CT) with excitation at 547 nm and emission at 573 nm. Previous sedimentation assays of the rhodamine-labeled actin indicated that 15% of the total fluorescence was unbound or bound to nonfunctional actin and this value was subtracted from all readings. The percentage of total actin bound to the fibrin clot was calculated as percent bound = (FL₀ - FL₁)/FL₀, where FL₀ is the initial fluorescence before fibrin polymerization and FL₁ is the fluorescence of solution minus fibrin clot.

Fibrinolysis experiments. Actin-containing fibrin clots were lysed by two different methods. In the first, fibrin, with or without actin, was polymerized by addition of a relatively high concentration of thrombin and a relatively low concentration of plasmin, chosen so that gelation was nearly complete before significant degradation of either fibrin or fibrinogen could occur, following the method of Shen et al.1 Fibrin gels were polymerized in a torsion pendulum and the rheologic properties of the clots measured during both clot formation and dissolution. The samples contained 3 g/L fibrin, 0.42 NIH U/mL thrombin, and 0.3 CU/mL plasmin in solutions containing 140 mmol/L NaCl, 10 mmol/L Tris-Cl, 2 mmol/L MgCl₂, 2.5 mmol/L CaCl₂, pH 7.4.

In the second method, fibrin clots (6 \( \mu \)mol/L) containing 6 \( \mu \)mol/L filamentous rhodamine-actin were formed in borosilicate glass culture tubes by the addition of thrombin. Clotting was initiated by the addition of thrombin (final concentration, 0.8 NIH U/mL). The clots were maintained at room temperature for 2 hours and then were gently removed from the tubes and soaked in an equal volume of buffer B (2 mmol/mL Tris, 0.5 mmol/L ATP, 2 mmol/mL CaCl₂, 0.2 mmol/L DTT, 150 mmol/L KCl, 2 mmol/L MgCl₂, pH 7.4) in the presence or absence of physiologic quantities of gelsolin (2 \( \mu \)mol/L). At various time intervals, the solution surrounding the clot was removed and the fluorescence was measured to determine the amount of rhodamine label released from the clot.

RESULTS

Viscoelastic properties of fibrin/actin gels. The presence of actin filaments during the polymerization of fibrinogen
Fig 1. Effect of F-actin on fibrin rheology. Increase in dynamic storage modulus ($G'$) after the addition of thrombin to fibrinogen, measured by oscillatory shear deformations of 2% strain amplitude and angular frequency 10 rad/s (1.6 Hz). The fibrinogen concentration was 2.0 mg/mL, and the actin concentration (in mg/mL) was 0 (○), 0.2 (△), 0.4 (▲), and 1.0 (●). All solutions contained 150 mmol/L NaCl, 2.2 mmol/L CaCl₂, 150 μmol/L ATP, 1.9 mmol/L MgCl₂, pH 7.4. G-actin was added to fibrinogen and 0.1 NIH U/mL thrombin was added 10 seconds later.

strongly affects the mechanical properties of the fibrin gel network. Figure 1 shows the effect on the shear modulus, a measure of the clot’s elastic resistance to deforming stresses, when increasing amounts of F-actin are incorporated into the clot. F-actin increases the shear modulus to an extent that depends on the actin concentration. The viscoelasticity of the actin filaments themselves may account for some of the increased shear modulus, but a significant component also results from the alteration of fibrin gel structure caused by inhibition of protofilament bundling previously described.19 The effect of F-actin is to make the clot finer, and finer clots might be expected to have lower shear moduli than coarse clots because they contain thinner filaments. However, fine clots may also exhibit higher shear moduli if the inhibition of fibrin bundle formation is coupled to an increase in the number of branch points,36,37 and higher shear moduli for finer clots have been reported for fibrin polymerized in the presence of IgG.4

One of the striking viscoelastic properties of fibrin gels is that they are strain-hardening; their elastic modulus increases with increasing amplitudes of deformation. Figure 2 shows that this feature of fibrin rheology, which may be crucial for its physiologic function as a pliable, but rupture-resistant hemostatic plug, is nearly completely eliminated by long F-actin filaments.

Not only does actin inhibit the strain-hardening of fibrin, it also reduces the elastic recovery of the clots. Figure 3A shows the nearly total reversibility in the shear modulus observed when a fibrin gel is first deformed in increments to large maximal strain and then strained to successively smaller deformations. The reproducibility of $G'$ after these large deformations reflects the high degree of elasticity of these gels and their resistance to mechanical failure even when highly deformed. The presence of even a low amount (0.2 mg/mL) of F-actin reduces the degree of strain-hardening and leads to irreversible decreases of approximately 40% in the shear modulus after straining, indicating that fibrin/actin gels are damaged by deformations in the range that could occur in vivo (Fig 3B). This phenomenon requires long actin filaments, because when the actin filaments are shortened by gelsolin, strain hardening is again observed, and the elastic recovery increases to levels near those of fibrin alone (Fig 3C).

**Fibrin-actin binding.** Binding assays showed that the amount of actin bound to fibrin clots depends on the length of the added actin filaments (Fig 4). In the absence of actin-shortening proteins, binding appears to be unsaturable, and the molar ratio of actin to fibrin in the clot is greater than 1:1 at the highest actin concentrations shown. This finding is due likely to the fact that actin filaments polymerized in the absence of specific actin-binding proteins generally attain filament lengths of several microns,38 and may not only bind to the fibrin clot but become entangled in it.19 When the filament length is limited by a 1:12 molar ratio of gelsolin (which results in gelsolin-capped filaments whose average length is 32 nm),31 binding appears to reach a limit at approximately a 2:3 molar ratio of actin to fibrin subunits (Fig 4). If actin is prevented from polymerizing by incubation of G-actin monomers with the actin monomer-binding protein DBP (Fig 4), the amount of actin bound to the clot is greatly decreased, but not entirely eliminated. Similar results were observed when actin was prevented from polymerizing by DNase I, which forms tight complexes with actin monomers but binds actin at a different site than DBP (data not shown). These results
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Fig 3. Shortening of F-actin by gelsolin reverses its effect on strain hardening and hysteresis of fibrin. The shear modulus \( G' \) is shown for measurements at 10 rad/s over a range of strain amplitudes beginning at 1% and increasing to 37% (A) or 30% (B and C) (C). After deformation at the maximal strain amplitude, measurements of \( G' \) were repeated at sequentially decreasing maximal strain amplitudes to ascertain the recovery of samples after deformation (C). The three panels show results for 2 mg/mL fibrin alone (A), fibrin plus 0.2 mg/mL F-actin (B), and fibrin plus actin oligomers (3:1 actin:gelsolin complexes) (C). Other conditions are given in the legend to Fig 1.

Fig 4. Binding of actin filaments and complexes to fibrin gels. Various amounts of rhodamine-labeled F-actin alone (O) or actin polymerized in the presence of DBP (●) or gelsolin (▲, ▴) were added to fibrinogen (2 mg/mL, 6 \( \mu \)mol/L) in T7 (100 mmol/L NaCl, 50 mmol/L Tris-Cl, pH 7.4) before clot formation by the addition of 1.7 NIH U/mL thrombin. The molar ratio of DBP to actin was 2:1, and the molar ratio of gelsolin to actin was either 1:2 (▲) or 1:12 (▲). The bound actin was removed from solution by winding the insoluble clot onto a glass pipette, and the amount of actin bound to the clot was measured from the difference between the fluorescence of the sample before and after clot removal, as described in the text.

Fig 5. Effect of \( \text{Ca}^{2+} \) and actin filament length on the binding of actin to fibrin. Actin was polymerized with various molar ratios of gelsolin to produce filaments of average lengths ranging from 34 nm to 2.7 \( \mu \)m. Polymerized actin (6 \( \mu \)mol/L) was mixed with 6 \( \mu \)mol/L fibrinogen in solutions containing 100 mmol/L NaCl and 50 mmol/L Tris-Cl, pH 7.4, either with (●) or without (○) 3 mmol/L \( \text{CaCl}_2 \), and the actin bound to fibrin after the addition of 1.7 NIH U/mL thrombin was measured as described in the text.

suggest that, while long actin filaments may be trapped nonspecifically in the forming fibrin clot, specific binding of short filaments and complexed monomers also occurs. This binding is judged to be specific because both short (32 nm) actin filaments and complexes of actin monomers and DBP bind to clots whose average pore sizes are several microns in diameter. The affinity of actin monomers or filament fragments for the fibrin gel is difficult to evaluate precisely because the presence of actin perturbs the structure of the matrix to which it binds, but the data of Fig 4 suggest that the affinity is sufficiently great (kd < 1 \( \mu \)mol/L) that it would be significant at the concentrations of fibrin and actin likely to occur in vivo.

The effect of filament length on the amount of F-actin incorporated in the clot is shown in more detail in Fig 5. In this experiment, a constant amount of actin (12 \( \mu \)mol/L; 0.5 mg/mL) was polymerized in the presence of various amounts of gelsolin and added to 6 \( \mu \)mol/L fibrinogen (2.0 mg/mL) before the addition of thrombin. Because each gelsolin nucleates and caps one filament, and under these solution...
conditions no uncapped filaments exist, the average number of actin subunits per filament equals the actin:gelsolin ratio, and the average length is calculated from the finding that there are 370 subunits in a micron of filament. By this calculation, the average length of actin filaments present in the solution of polymerizing fibrinogen ranged from 34 nm to 2.7 \mu m. More than 80\% of the total actin bound to the clot when the average filament length was very long, but even very short actin filaments, formed at high gelsolin: actin ratios, bound significantly. Moreover, the binding of filaments shorter than 300 nm did not depend on their length, suggesting that the interaction is not due entirely to trapping of filament within the pores of the fibrin gel, but to a specific interaction between actin and fibrin. These results confirm that actin binds to fibrin in part independently of actin ratios, bound significantly. Moreover, the binding of F-actin to fibrin does not depend on changes in fibrin structure caused by millimolar calcium ion concentrations.

**Fluorescence microscopy.** Confocal microscopy of actin-containing fibrin clots was performed to examine directly the interaction of actin with fibrin. Figure 6a shows a typical fluorescence image obtained when rhodamine-labeled F-actin was added to a fibrinogen solution before clot formation; Fig 6b shows the corresponding phase contrast image of the fibrin strands. The fluorescence of the labeled F-actin coincides with the structure of the fibrin network, demonstrating the direct association of rhodamine-actin with fibrin. Similar images were obtained using fluorescein-labeled F-actin (data not shown). The coincidence of F-actin and fibrin strands suggests that these polymers associate laterally and do not interact entirely by a purely nonspecific steric overlap. When the actin filament length is reduced to 32 nm by gelsolin, the fluorescent labeling of the fibrin network is much weaker (Fig 6c) and some patches of bright fluorescence corresponding to spots of high density in phase contrast (Fig 6d) are seen. These patches may represent sites at which short actin filaments bind or are trapped in the clot. The F-actin network in the absence of fibrin cannot be visualized by confocal microscopy because the thin (9 nm) actin filaments formed under these conditions do not form bundles and therefore produce a network too fine to be resolved using confocal microscopy (Fig 6e and f). Incubation of rhodamine-actin with DBP before clot formation significantly reduced the intensity of the fluorescent labeling of fibrin, but a distinct pattern of fluorescence coincident with the fibrin strands was still visible (Fig 6g). These results are consistent with the data in Figs 4 and 5, which showed reduced, but still measurable binding of complexed actin monomers to fibrin clots. The specificity of the binding of F-actin to fibrin is supported by the finding that when fluorescein-avidin is added to a fibrinogen solution instead of rhodamine-labeled F-actin before clot formation, fluorescent labeling of the fibrin strands does not occur (Fig 6h).

**Effect of actin filaments on lysis of fibrin clots.** Incorporation of actin into a fibrin clot results in inhibition of its rate of lysis by plasmin, as measured by the release of radioactive fibrin fragments, which is most obvious after several hours. Because the loss of a clot’s structural integrity (as reflected in its elastic modulus) is one of the first consequences of lysis, we performed the experiments shown in Fig 7 to determine if actin’s effect upon clot lysis could be detected at an earlier time. The incorporation of long (Fig 7A) and short (Fig 7B) actin filaments into fibrin gels retarded both the increase in shear modulus during clot formation and the plasmin-induced decrease in shear modulus during lysis. The effects of incorporated (both fibrin-bound and sterically trapped) actin filaments was evident 20 minutes after lysis began. Thus, actin incorporation into a clot results in a more rapid change in the physical properties of the clot under lytic conditions than is reflected by the rate of release of fibrin fragments into the lysis bath. Control viscoelastic measurements verified that F-actin itself was not degraded by plasmin during the time course of these experiments (data not shown).

Because it is of potential therapeutic importance to know whether actin filaments trapped in fibrin clots may be eluted from the clot, actin-containing clots were incubated in the presence or absence of plasma gelsolin. As shown in Fig 8, incubation of clots containing rhodamine-actin in physiologic (2 \mu mol/L) concentrations of plasma gelsolin resulted in a loss of most of the actin from the clots. Because the fluorescence of rhodamine-labeled F-actin is higher than that of free rhodamine or rhodamine-actin monomers, the amount of label released into the medium may be somewhat underestimated by the fluorescence of the medium compared with that of the initial labeled F-actin solution. The amount of labeled actin that remains clot-associated is consistent with the results shown in Fig 2. Further, elution of actin from the clots restored their susceptibility to plasmin-mediated lysis, as judged visually. Inspection after 18 hours showed macroscopic remnants of those clots that had not been exposed to plasma gelsolin, while clots incubated in solutions containing plasma gelsolin were totally dissolved. The loss of clot-associated rhodamine does not reflect total clot lysis, but rather the known cleavage of the rhodamine-labeled terminal dipeptide of actin by plasmin.

**DISCUSSION**

Hemostasis depends on a balance between the formation and dissolution of clots composed of fibrin networks and the varying proportions of other plasma proteins and blood cells. These networks form by the polymerization of fibrin monomers into protofibrils, and by the subsequent lateral association of protofibrils into bundles, which takes place in vivo in a complex (and potentially variable) mixture of other proteins. Because fibrin clot formation is sensitive to the effects of macromolecules that impede the diffusion of the growing polymer strands, a number of physiologically important molecules, such as IgG, fibronectin, albumin, glycosaminoglycans, etc., alter the structure of fibrin gels. Many of these substances are normal constituents of plasma, and may explain in part the recognized differences between plasma clots and those formed from purified fibrinogen. Studies of these effects on fibrin clots have suggested mechanisms whereby inflammatory events lead to alterations in the process of blood coagulation.
Fig 6. Confocal fluorescence (a, c, e, g, and h) and phase contrast (b, d, and f) images of actin-containing fibrin clots. Addition of rhodamine-labeled F-actin to fibrinogen before thrombin-induced clot formation resulted in fluorescently labeled filaments (a). The corresponding phase contrast image (b) shows that the fluorescence of labeled actin colocalizes with the fibrin strands. Incubation of rhodamine-actin with gelsolin (c and d) before clot formation resulted in reduced fluorescent labeling of the fibrin network. In the absence of thrombin the fluorescent actin filaments cannot be resolved with the confocal microscope (e). The corresponding phase contrast image (f) shows that a fibrin clot has not formed. Inhibition of actin polymerization by DBP resulted in reduced fluorescent labeling of fibrin strands (g). The specificity of the interaction of actin with fibrin is demonstrated in (h), which shows the absence of fluorescent fibrin when fluorescein-avidin was added to fibrinogen before thrombin-induced clot formation. (c) and (g) correlate with data showing reduced binding of actin monomers complexed with gelsolin and DBP to fibrin gels (see Figs 4 and 5). The bar in (h) is equal to 9 μm for all panels.
However, clots that form at sites of tissue injury are likely to be different from those prepared from normal plasma, and may contain proteins released from platelets, white blood cells, endothelial cells, or other types of injured cells, as well as acute-phase proteins. While much previous investigation of the bleeding and thrombotic tendencies noted in patients suffering from different forms of tissue injury has focused on the dysregulation of the clotting and fibrinolytic systems, collectively known as disseminated intravascular coagulation, little consideration has been given to the structure and function of the clots that form in such patients, which could be altered in clinically significant ways. Conformational changes in fibrin clots, for example, may render them more susceptible to disruption by mechanical forces, whether found naturally in the vascular tree or imposed by medical devices such as membrane oxygenators or mechanical ventilators. Alternatively, alterations in clot structure may affect their sensitivity to the fibrinolytic protein plasmin, rendering them resistant to plasmin’s action in a manner analogous to that reported for a patient with a congenital dysfibrinogenemia. The possibility that actin and perhaps other intracellular proteins released during tissue trauma may exert these effects is suggested by the finding that material released from platelets alters both the structure and lysis of fibrin clots in vitro.

Actin has previously been shown to interact with fibrin and may be cross-linked to it in vitro by the action of factor XIIIa. We have previously reported that actin filaments alter fibrin clot structure, resulting in the formation of fine, less turbid clots. The addition of gelsolin, a protein that shortens actin filaments, abrogates this effect, indicating that actin filaments intertwine with fibrin fibrils, inhibiting their lateral association. The results reported here extend these studies by reporting the effects of actin filaments on the mechanical properties of actin-containing clots and by examining the effects of the other actin-binding protein of plasma, DBP, on actin/fibrin interactions.

Our results show that there are two types of actin-fibrin interactions. A relatively nonspecific interaction is due to trapping of actin filaments within the fibrin gel, and a more specific type of binding occurs even when actin filaments are shorter than the average pore size of the fibrin gel. The addition of DBP, which binds to actin monomers and prevents them from polymerizing into actin filaments, has an effect upon the amount of actin bound to clots that resembles the effect of plasma gelsolin. Both proteins inhibit binding, supporting the idea that fibrin clots trap actin filaments. Depression of the plasma content of either protein in the vicinity of a forming clot would therefore probably result in increased amounts of clot-associated
actin. That some actin binds to fibrin even when equimolar concentrations of these actin-binding proteins are present is not surprising, because a specific interaction between actin and fibrin is implied by the cross-linking of actin to fibrin by factor XIIIa.47

The rheologic properties of actin-containing clots differ from those of pure fibrin clots, as would be expected when two interdigitating filament networks are mixed together. Previous workers have shown that the rheologic properties of actin and fibrin networks differ both quantitatively and qualitatively, and the viscoelasticity of a mixture of two such polymers is difficult to predict. These experiments show that incorporation of actin into a fibrin clot leads to a disappearance of one of its unusual properties, strain-hardening, and lessens its ability to withstand large deformations without structural change. Clots containing actin are thus more brittle than those without. Addition of gelsolin, which solates the actin network, prevents this effect, suggesting that the brittleness of actin/fibrin clots is due more to steric interactions between the two filament types than to direct actin/fibrin binding. In short, actin can be incorporated into fibrin clots without compromising their physical properties as long as the filaments are not too long.

Gelsolin thus exerts a protective effect upon fibrin clots when added to actin before the fibrin clot is formed. By shortening actin, gelsolin decreases the amount of actin associated with the clot and maintains the clot’s rheologic properties. In addition, gelsolin is able to shorten actin filaments trapped in a fibrin clot, allowing much of the clot-associated actin to diffuse from the clot. These experiments also confirm that clot-associated actin inhibits plasmin-mediated fibrinolysis. Removal of actin from a clot by plasma gelsolin can thus be considered to be beneficial insofar as it aids in the restoration of the homeostatic mechanisms that regulate clot formation and dissolution.

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