Thiol Oxidation of Actin Produces Dimers That Enhance the Elasticity of the F-Actin Network

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ABSTRACT Slow oxidation of sulfhydryls, forming covalently linked actin dimers and higher oligomers, accounts for increases in the shear elasticity of purified actin observed after aging. Disulfide-bonded actin dimers are incorporated into F-actin during polymerization and generate cross-links between actin filaments. The large gel strength of oxidized actin (>100 Pa for 1 mg/ml) in the absence of cross-linking proteins falls to within the theoretically predicted order of magnitude for uncross-linked actin filament networks (1 Pa) with the addition of sufficient concentrations of reducing agents such as 5 mM dithiothreitol or 10 mM β-mercaptoethanol. As little as 1 gelsolin/1000 actin subunits also lowers the high storage modulus of oxidized actin. The effects of gelsolin may be both to increase filament number as it severs F-actin and to cover the barbed end of an actin filament, which otherwise might cross-link to the side of another filament via an actin dimer. These new findings may explain why previous studies of actin rheology report a wide range of values when purified actin is polymerized without added regulatory proteins.

INTRODUCTION

A dynamic network of actin filaments (F-actin) is one of the most prevalent features of the cytoskeleton. In the leading lamella of locomotive cells such as fish keratocytes and macrophages (Small, 1994; Stossel, 1993), a dense protein gel consisting primarily of F-actin not only helps support the structure but also allows for shape changes that are essential for motility and function. For instance, the protrusive force of a macrophage phagocytosing a bacterium depends on how strong and dynamic its cytoskeletal network is, which is due largely to the dynamic assembly and cross-link of the actin network. A dense actin network, loosely defined as an actin gel, is also found in nonmotile cells, and the elasticity of such a network at least partially determines the overall mechanical strength of the cells. Therefore, accurate measurements of the mechanical properties of F-actin are useful for developing quantitative models of cell motility and mechanics that consider the viscoelasticity of the cell cortex and ascertaining the mechanical effects of specific actin-binding proteins.

Solutions of actin filaments formed under conditions that limit their length to a few microns behave rheologically as viscous liquids or very soft viscoelastic materials with elastic (or storage) moduli on the order of 1 Pa at 1 mg/ml protein concentration (summarized in Xu et al., 1998a). Such low elastic moduli, even when extrapolated to the much higher actin concentration of the cytoplasm, appear to be too low to account for cellular elasticity values (Lo et al., 1998; Glogauer and Ferrier, 1998). Actin filament cross-linking proteins such as α-actinin, gelation factor/ABP120, or filamin/ABP280, which have large effects on actin network rheology (Goldmann et al., 1997; Janmey et al., 1990; Janssen et al., 1996; Ruddies et al., 1993a; Stossel, 1984; Wachsstock et al., 1993; Xu et al., 1998b), best explain the measured cell elasticity. Consistent with classical theories of polymer gelation (Ferry, 1980), the addition of a critical concentration of cross-linkers greatly increases the elastic resistance of actin networks, with values exceeding 100 Pa for 1 mg/ml actin cross-linked by ABP280 or for avidin cross-linking of biotinylated actin filaments (Janmey et al., 1990; Wachsstock et al., 1993).

In contrast to the approximately consistent and reproducible results for actin filaments of regulated length and cross-links, measurements of the rheology of highly purified actin in the absence of other proteins have yielded widely varying results ranging from <0.1 Pa to >100 Pa (Janmey et al., 1994; Xu et al., 1998a). Based on the effects of actin filament-severing proteins, this discrepancy has been attributed in some studies (Janmey et al., 1994) to slight differences in filament length, which cannot accurately be controlled because of the nucleation-elongation mechanism of actin polymerization. On the other hand, because different actin preparations have different rheological properties without a discernible difference in filament length (Xu et al., 1998a), a primary role for filament cross-linking has also been proposed, but the cross-linking species, whether a contaminating protein or a modification of actin, has not been identified.

Recently, a collaborative effort tested many variables of different methods to prepare actin and measure the viscoelasticity of F-actin, including the rheologic instruments used, preparation of acetone powder, protein extraction protocols, gel filtration, storage of the purified actin by rapid freezing, and polymerization before the rheological mea-

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surements (Xu et al., 1998a). The total effects of these factors account for a range of variation of not more than a fewfold. In contrast, allowing the G-actin to age at 4°C without dialysis in freshly prepared buffers increased the storage modulus by more than an order of magnitude. Such an interesting finding by Xu et al. strongly suggests that certain slow changes in solution chemistry may drastically affect the rheology of the actin network. However, the primary factor responsible for this large change was not identified. The present study provides evidence that the factor is slow oxidation of G-actin, producing oligomeric actin species with filament cross-linking activities. Actin filaments protected from oxidation have measured moduli on the order of 1 Pa for 1 mg/ml. This value is consistent with predictions of recent theoretical models of viscoelasticity of semiflexible polymer networks (Hinner et al., 1998; Maggs, 1997; Morse, 1998b).

MATERIALS AND METHODS

Proteins

Acetone powder of rabbit skeletal muscle was prepared following the protocol of Eichinger et al. (1991), which was described as "preparation B" by Xu et al. (1998a). Multiple preparations of actin have been performed according to the procedure of Spudich and Watt (1971). In selected preparations, a gel filtration step was added by using a Sephadex G-150 column, and the procedure was essentially the same as described by Pardee and Spudich (1982). The nonpolymerizing actin solution contained 2 mM Tris-Cl buffer at pH 8.0, 0.2 mM $CaCl_2$, 0.5 mM ATP, 0.2 mM dithiothreitol (DTT), and 0.5 mM NaN_3. Actin was polymerized by 2 mM $MgCl_2$ and 150 mM KCl. Pyrene-labeled actin was prepared by the method of Kouyama and Mihashi (1981). Human plasma gelsolin was prepared by the method of Kurokawa et al. (1990). Turkey gizzard α-actinin was prepared by a published method (Feramisco and Burridge, 1980) and was a kind gift of Dr. Barbara Leinweber at Boston Biomedical Research Institute.

Oxidation of actin

Two samples of the same actin preparation were added to separately sealed chambers immediately after the final dialysis and centrifugation step of the purification. One sample was exposed to a gentle stream of air, and the other to a stream of pure N_2. At various times, aliquots were taken from each sample and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and falling ball viscometry. In other preparations of actin, the samples were handled by the methods described by Xu et al. (1998a).

Rheology

Rheological measurements were made with a Rheometrics RFS II instrument (Rheometrics Scientific, Piscataway, NJ) with cone-and-plate geometry. A typical series of measurements for one sample included monitoring the shear moduli at 1% strain and a frequency of 10 rad/s, during actin polymerization for 2 h, followed by measurements of its frequency dependence, stress relaxation of the network after a 2% strain, and detection of the gel collapse by application of sequentially increased strains.

Low-speed sedimentation

Two aged actin samples, one with and the other without incubation with 10 mM DTT for 30 min, were polymerized for 2 h by the addition of 2 mM $MgCl_2$ and 150 mM KCl. The two samples were then centrifuged at $14{,}000 \times g$ by a tabletop centrifuge. After intervals of 10, 20, or 30 min of centrifugation, ~80% of the solution was gently taken from the top of each test tube, and the actin concentration was measured by a spectrophotometer, using an extinction coefficient of 6.6 at 290 nm for 1% F-actin. The samples were then put back in their original test tubes and subjected to more centrifugation.

Light scattering assay

The light scattering intensity of F-actin solutions was monitored by a previously described method (Tang and Janmey, 1996).

SDS-gel electrophoresis

Polyacrylamide gels (10%) were used to characterize protein purity after each actin preparation. The protein samples were denatured for electrophoresis with and without a reducing agent such as β-mercaptoethanol (BME) or DTT to detect protein complexes that are connected by disulfide bonds. The common step of heating the samples for a few minutes at 95°C was also omitted, to avoid breaking intermolecular disulfide bonds in the latter case.

Fluorescence imaging

The fluorescence technique for visualizing actin filaments labeled with TRITC-phalloidin (Molecular Probe) has been described previously (Käs et al., 1996). The only modification of this work was the omission of BME from the antibleaching buffer so that interfilament interaction could be observed in weakly reducing actin buffer solutions.

Polymerization assay

Actin polymerization was detected by measurement of changes in pyrene fluorescence by the method of Kouyama and Mihashi (1981), using a Perkin-Elmer LS-5B luminescence spectrometer. The excitation wavelength was set at 365 nm, and the emission was detected at 386 nm. The sample contained 1 μM pyrene-labeled actin and 4 μM unlabeled actin. Polymerization was initiated by adding 2 mM $MgCl_2$ and 150 mM KCl, followed by brief mixing immediately before the fluorescence intensity was recorded.

RESULTS

Initial observations on the rheology of actin from various preparations

Fresh actin samples from several independent preparations polymerize to form weak viscoelastic gels, with values of storage modulus (G') on the order of 1 Pa. Samples rapidly frozen by liquid nitrogen, stored at −80°C, and thawed gave more variable G' values; the variation correlated with the number of days that had elapsed between the start of actin extraction from acetone powder and the time when the samples were frozen in liquid nitrogen, and the general trend is that the G' value increases with aging. These observations are consistent with those reported by Xu et al. (1998a), including the finding that the rates of change are variable, even among similar preparations (data not shown).

Exposure of G-actin to air increases the elasticity of F-actin and produces oligomeric actin under nonpolymerizing conditions

To test a hypothesis that oxidation of actin may cause changes in viscoelastic properties of actin, rheological measurements were performed for actin samples exposed to either air or pure N_2 for several days. Fig. 1 shows by falling ball viscometry that the apparent viscosity of an actin network increases rapidly with aging if the sample is exposed to a constant stream of air, but not if it is exposed to N_2. When the actin exposed to air was analyzed by SDS-PAGE without reducing agent added to the sample buffer, a significant amount of actin dimer was detected, in contrast to the control actin sample exposed to N_2, which contained only actin monomers (*inset* in Fig. 1). These results suggest that oxidation during prolonged exposure of G-actin to air may account for the difference in rheology when differently prepared samples are polymerized and that formation of actin dimers and smaller amounts of higher oligomers is linked to the increased elasticity of aged actin.

Excess reducing agent decreases the storage modulus of aged actin to the level of fresh F-actin

The addition of reducing agents, as shown in Fig. 2, lowers the high elastic modulus of an actin sample that had been allowed to stand in an oxidizing environment to the value of a fresh sample. The actin used in this study was thawed from a preparation stored for 5 days before freezing. Immediately after thawing at 37°C and polymerization by the addition of 2 mM $MgCl_2$ and 150 mM KCl, this sample of actin had an elastic modulus of ~25 Pa (*curve with open circles*). The addition of 2 mM BME 2 h before polymerization decreased the elastic modulus significantly, but higher concentrations of reducing agent (5 mM DTT or 10 mM BME) were required to lower the modulus to that of fresh F-actin. A reproducible but unexplained transient overshoot was observed at moderate levels of reducing agents (2 and 5 mM BME, or 2 mM DDT), even with overnight incubation. The addition of excess phalloidin (2:1 molar ratio to actin) accelerated gel formation but did not affect the final gel strength.

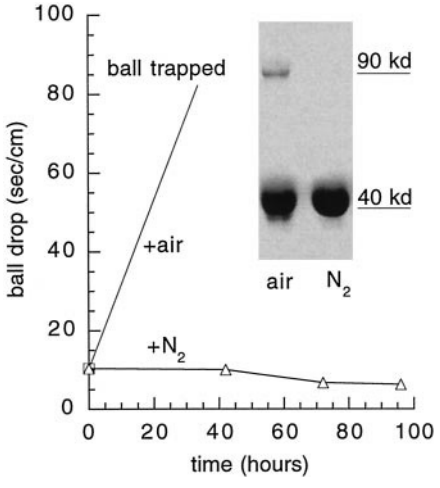

FIGURE 1 Falling ball viscometry monitoring the changes of actin with constant streams of either air (□) or N_2 (△). The numbers on the ordinate indicate the time in seconds for the ball to drop by 1 cm, which is proportional to the apparent viscosity of the actin network. (*Inset*) Detection of actin and actin dimers by SDS-PAGE in the absence of reducing agent. The left lane shows, for the sample treated by air, a 90-kDa band, in addition to the 42-kDa actin band, and the right lane shows for the control sample without oxidation a single band of monomeric actin.

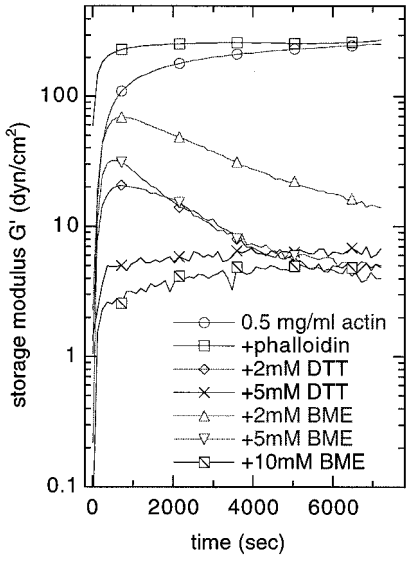

FIGURE 2 The storage modulus G' monitored during the polymerization of actin thawed from a preparation kept at 4°C for 3 additional days before freezing. Curves from top to bottom include actin +20 μM phalloidin (□), actin control (○), + 2 mM BME (△), +5 mM BME (▽), +2 mM DTT (◇), +5 mM DTT (+), and +10 mM BME (□), respectively. The actin concentration was 0.5 mg/ml for all samples.

F-actin polymerized from aged actin sediments as aggregates or a cross-linked network

Fig. 3 compares the sedimentation profile of the polymerized aged actin with and without the addition of 10 mM

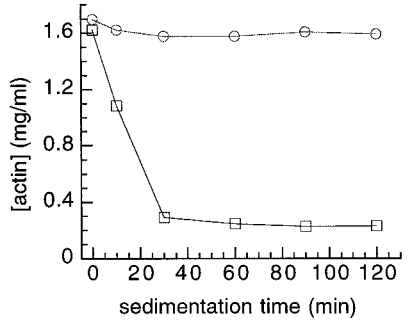

FIGURE 3 Concentration of F-actin remaining in the supernatant after various durations of low-speed centrifugation. The circles are for the polymerized aged actin pretreated with 10 mM DTT, and the squares represent aged F-actin without additional DTT.

DTT. Control experiments suggest that fresh F-actin does not sediment after 2 h of centrifugation at 14,000 × *g* (data not shown). The actin network formed from the aged actin sedimented within 30 min of centrifugation, in contrast to an identical sample pretreated with 10 mM DTT, which did not sediment (Fig. 3). On the other hand, no significant difference in light scattering was detected between such samples of aged actin with and without DTT treatment. These results suggest that the aged actin contains cross-linked filaments but not large aggregates of F-actin, and the cross-linked actin sedimented at low gravitational acceleration because of the collective response of the network. The curve in Fig. 3 without DTT treatment also shows that ~20% of actin filaments did not sediment with the network even after prolonged centrifugation, suggesting that this fraction of actin existed as filaments not integrated into the network.

Formation of highly elastic actin gels correlates with the presence of covalently cross-linked actin dimers and oligomers that are dissociated by reducing agents

The large effect of a reducing agent on F-actin elasticity strongly suggests that a disulfide bond formed between two actin monomers may be responsible for the cross-linking of F-actin, which would increase the storage modulus G'. Analysis of actin preparations by SDS-PAGE with and without BME or DTT (Fig. 4) shows that the protein species migrating at higher molecular weights than G-actin disappeared when 10 mM DTT was added to the G-actin solution before electrophoresis. A complete survey of several actin samples showed that those samples that had high shear moduli also had detectable DTT-sensitive actin oligomers, and samples with low moduli did not.

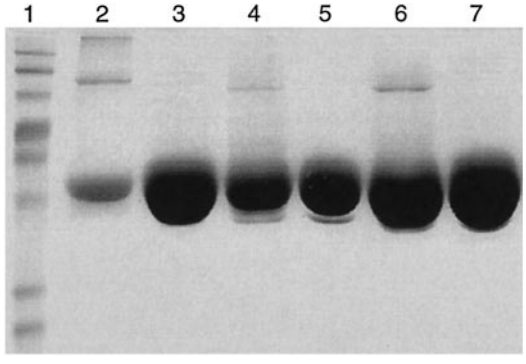

FIGURE 4 SDS-PAGE of two preparations of actin with and without 10 mM DTT. *Lane 1*: Gibco molecular weight markers with reference molecular masses from top down: 220, 130, 90, 70, 60, 40, 30, 20 kDa. *Lane 2*: Mixture of turkey gizzard α-actinin (1 μg, molecular mass = 110 kDa) and actin (4 μg). *Lane 3*: Actin preparation 1, frozen by liquid nitrogen while fresh and later thawed for SDS analysis. *Lanes 4* and *5*: Another preparation of aged actin without (*lane 4*) and with (*lane 5*) DTT. *Lanes 6* and *7*: Preparation 1 aged for 4 days and analyzed without (*lane 6*) and with (*lane 7*) 10 mM DTT.

The amount of disulfide-linked actin oligomers was not altered after actin polymerization, as determined by SDS-PAGE of F-actin samples. Actin dimers were also detected in F-actin separated from unpolymerized actin by centrifugation, suggesting that the oxidized actin dimers were incorporated into the actin filaments (data not shown). In an attempt to correlate the transient overshoots in G' at moderately reducing conditions with a transient increase in the level of actin dimers during polymerization (shown in Fig. 2), SDS-PAGE was performed with samples taken at several times during polymerization with 2 mM DTT. No corresponding transient increase in the amount of dimers was detected, possibly because of the limited sensitivity of SDS-PAGE (data not shown).

The viscoelasticity of the actin gels with a high elastic modulus is characteristic of a cross-linked network

The aged actin in low concentrations of reducing agent not only had high G' values when compared to other samples at the same frequency and amplitude of oscillatory deformation, but the whole spectrum of rheological properties is characteristic of a cross-linked network (Fig. 5). The G' value is virtually constant in the measured range of frequency between 0.1 and 100 rad/s (Fig. 5 *A*). During stress relaxation measurements at 2% strain, the initial shear modulus of 20 Pa relaxes only slightly after more than 30 min (Fig. 5 *B*). Such a gel also exhibits prominent strain hardening behavior and gel collapse, which occurs typically at ~10% strain (Fig. 5 *C*). These results are consistent with the previously published values for pure actin in the cases in which high G' values were detected (Janmey et al., 1994), as well as for actin cross-linked by ABP280 (Janmey et al., 1990).

The protein concentration dependence of G' was tested for three different preparations of actin that gave large G' values. All samples display a rather steep concentration dependence to a power greater than 2 (Fig. 5 *D*), which fits theoretical predictions for cross-linked networks (MacKintosh et al., 1995; Satcher and Dewey, 1996). In contrast, the theoretically predicted exponent for an uncross-linked network of semiflexible polymers such as F-actin is 1.4 (Hinner et al., 1998; Maggs, 1997; Morse, 1998b).

Actin networks with high elasticity contain stable end-to-side filament connections that can be eliminated by reducing agents

To visualize the possibly different morphology of F-actin formed from the aged actin, we labeled the actin filaments with rhodamine phalloidin and observed the fluorescence images of labeled filaments at nanomolar concentrations. In contrast to previous studies that found no evidence for filament-filament bonds, in this study we omitted BME from the antibleaching buffer. A solution of labeled F-actin

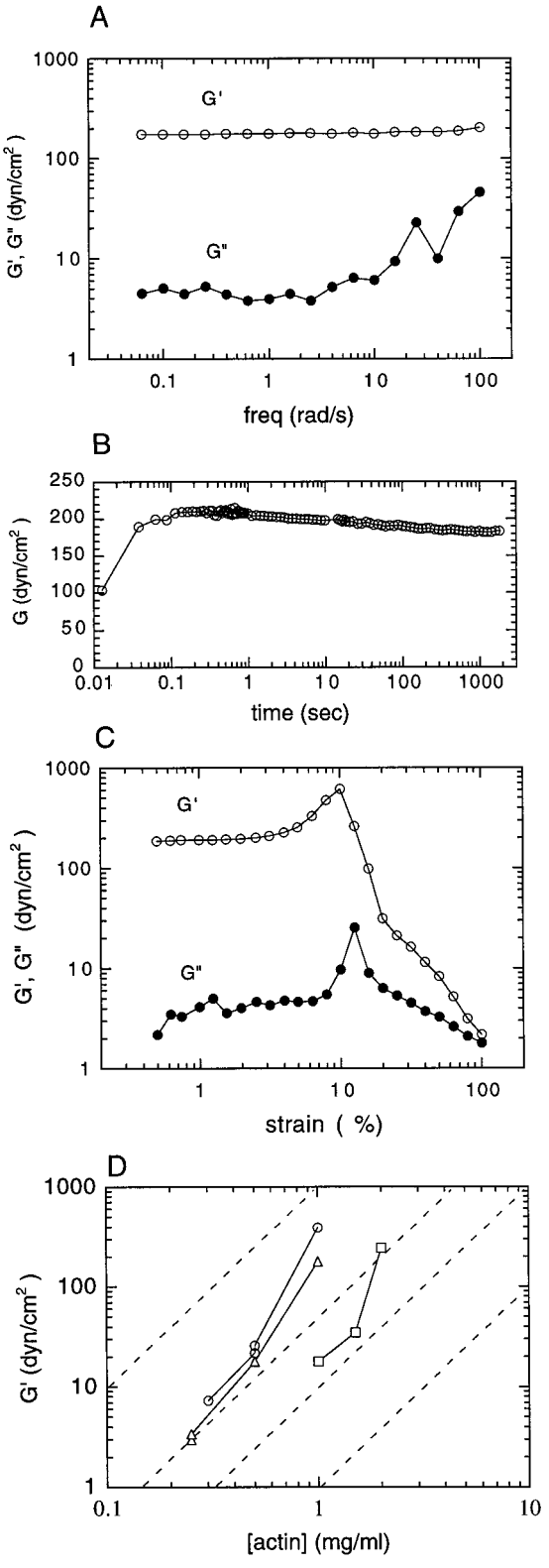

FIGURE 5 Rheological properties of a typical actin gel formed by polymerizing aged G-actin as described in Fig. 2. (*A*) Frequency dependence. (*B*) Stress relaxation curve following a 2% strain. (*C*) Strain dependence for storage (G') and loss (G'') moduli. (*D*) Concentration dependence for three preparations of actin including the one shown in Fig. 2 (△).

polymerized from aged G-actin unfortified with reducing agents contained a significant number of end-to-side-associated actin filament pairs (Fig. 6 *B–F*), in contrast to the same actin treated with BME after polymerization (Fig. 6 *A*), in which case such configurations were very rare. Images of single filaments similar to Fig. 6 *A* were observed with samples of fresh actin untreated with BME (not shown). Comparison of the filaments illustrated in Fig. 6 shows that the addition of reducing agents did not significantly reduce the average filament length, suggesting that the much higher storage modulus of F-actin in samples containing low concentrations of reducing agent was not due to a much larger average filament length.

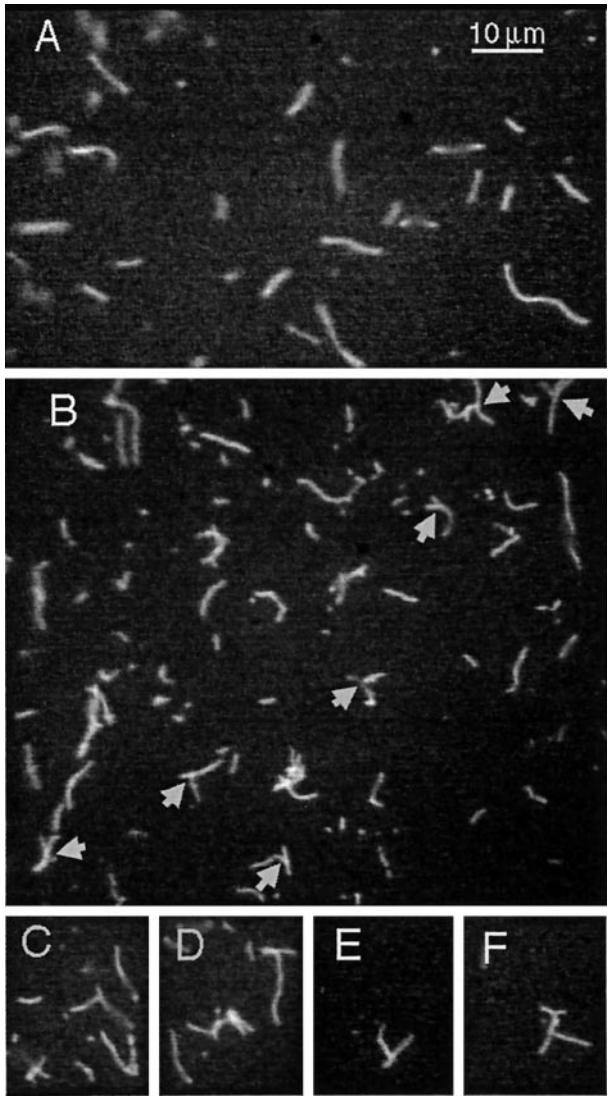

FIGURE 6 Fluorescent images of F-actin with (*A*) and without (*B–E*) 1% BME. Most of the filaments move rather fast because of thermal motions, but the filaments in the images are those stuck to the glass surface. The arrowheads in *B* point at "T-shaped" end-to-side cross-linking sites. A few more examples of end-to-side cross-links from other optical fields are shown in *C–E*.

Gelsolin has a large effect on the elasticity of the cross-linked actin network, but actin polymerization kinetics are unaffected by the presence of actin dimers

Consistent with previous reports, the G' values for the aged pure actin gel were strongly reduced by gelsolin (Fig. 7 *A*). A gelsolin:actin ratio of 1:1500 reduced G' by at least an order of magnitude, and 1:500 gelsolin:actin reduced the G' value to below 1 Pa, comparable to fresh actin regulated by the same amount of gelsolin (data not shown).

The potential effect of oxidized actin oligomers on actin polymerization kinetics was tested without and with gelsolin (Fig. 7 *B*). In the control samples, G-actin was incubated for over 30 min with 10 mM DTT to ensure that no actin dimers and oligomers were present during polymerization. Actin dimers and trace amounts of oligomers did not significantly affect the polymerization kinetics, suggesting no nucleation or capping activity of these oxidized species. The nucleation function of gelsolin was also unaffected by the actin dimer, suggesting that it is unlikely to bind gelsolin in a way that alters the nucleation activity of gelsolin molecules.

DISCUSSION

The present study confirms a previous report from Xu et al. (1998a) that the variability in rheologic measurements of actin depends on slight variations in the preparation of actin that are not obvious from analysis by SDS-PAGE, electron or light microscopy, or polymerization kinetics. Our studies further indicate that the primary factor responsible for these differences is not a contaminating protein, but a variable and small amount of disulfide-bonded actin dimers and/or oligomers that create cross-links when incorporated into filaments.

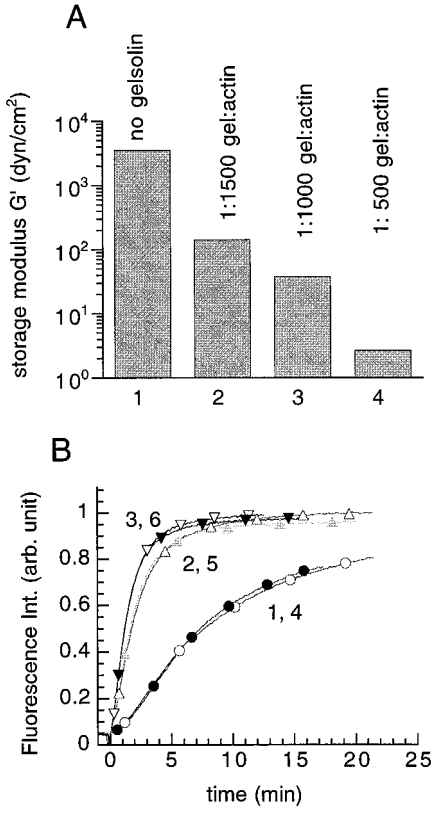

FIGURE 7 (*A*) Effects of gelsolin on G' for an aged actin stock. (*B*) Polymerization kinetics of aged actin with (*curves 1, 2, and 3, solid symbols*) and without (*open symbols*) 10 mM DTT. The samples contained 0 nM (1, 4), 20 nM (2, 5), and 50 nM (3, 6) gelsolin, respectively.

The apparent molecular mass of the disulfide-bonded dimers is ~90 kDa, similar to that of the lower dimer (LD) detected as a transient species occurring during actin polymerization (Millonig et al., 1988; Steinmetz et al., 1997) and stabilized by zero-length chemical cross-linkers. However, neither the previously described upper or lower actin dimers were formed by disulfide bonding. Therefore, we propose that the disulfide-bonded actin dimers (DSDs) may be a distinct structure. Intra- and intermolecular disulfide bonds have previously been detected in actin. Disulfide-dependent formation of actin aggregates that increased sample birefringence was first reported by Ishiwata (1976), and a dimeric species bound at Cys^{374} and similar to the lower dimer was found to be promoted by gelsolin (Hesterkamp et al., 1993). The latter report showed that the complex of lower dimer with gelsolin did not nucleate filament assembly. We found that the DSD did not inhibit the ability of gelsolin to accelerate actin polymerization. Such a result suggests that either gelsolin does not bind DSD more favorably than actin monomers, or that gelsolin does preferentially bind DSD, but gelsolin and gelsolin/DSD complexes have comparable nucleating activities. In conclusion, these various findings suggest that multiple forms of disulfide-bonded actin dimers exist.

The transient overshoots in storage modulus (G') during polymerization occurred reproducibly when moderate amounts of a reducing agent were added (1–5 mM BME or 0.5–2 mM DTT; see Fig. 2). Such a phenomenon is not likely to be due to a slow kinetics for competitively reducing the disulfide bonds by mass action, because the samples incubated with the added reducing agent overnight were found to show similar overshoots during polymerization. One possiblity is that the transient rise of the previously characterized LDs may act to enhance the gel elasticity by forming temporary branches of actin filaments (Steinmetz et al., 1997). Such LDs would not be detected by the SDS analysis in this report, because the samples were not treated with an external cross-linking reagent. It remains unknown at present if the transient LDs affect the elasticity of a polymerizing actin network, and why the effect only manifests itself in a moderately reducing environment.

A hypothesis for how DSDs form interfilament cross-links is shown in Fig. 8. Assuming that the DSD is linked via Cys^{374}-Cys^{374}, the packing of actin monomers in the Heidelberg model of F-actin (Holmes et al., 1990; Lorenz et al., 1995) would allow incorporation only at the barbed end, and hence a growing actin filament with its barbed end capped would not be able to form links to another filament at the site of a DSD. Accordingly, the solation effect of

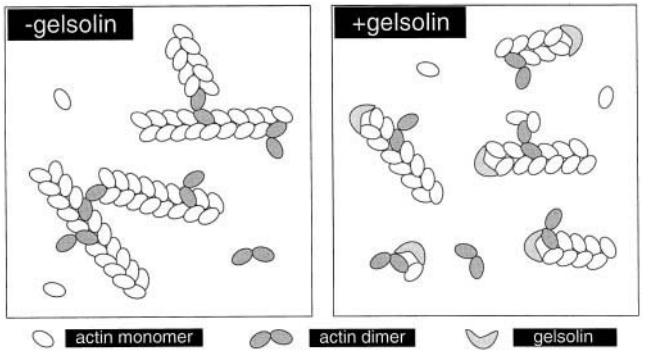

FIGURE 8 A simple model of end-to-side cross-linking of F-actin with DSD incorporated along the filaments (*left*). A few possible configurations are shown when gelsolin is added (*right*).

gelsolin could result in part by regulating the filament length due to its known nucleating and severing activities, but also by capping the barbed ends and hence preventing their attachment to the side of other actin filaments via DSDs. Another possibility is that gelsolin interacts directly with a DSD, sequestering it from a monomer pool (also illustrated in the scheme on the right side) and removing these species from the polymerizing pool. However, DSD in the present study does not affect the nucleation activity of gelsolin (Fig. 7 *B*), suggesting that either it does not have significantly greater affinity for gelsolin than unmodified actin, or that gelsolin and gelsolin/DSD complexes have comparable nucleating activity.

Disulfide-mediated cross-linking as the cause of large moduli sometimes reported for F-actin is consistent with the fact that the discrepancy in the storage modulus is nearly eliminated when the filaments are relatively short. In the simplest model for actin filament cross-linking, which adequately describes the sol-gel transition of ABP-cross-linked F-actin (Brotschi et al., 1978), an average of two links per filament is required for gelation to occur. Because even extensively oxidized G-actin contains a limited concentration of cross-linking actin species, making the filaments shorter will shift the balance between filament number and cross-linker concentration to the point where a network cannot form. Therefore, the largest variance is expected if the average filament length is large enough so that cross-linking by a low percentage of the oxidized species suffices for gelation.

The experimental findings that uncross-linked actin networks of 1 mg/ml long actin filaments have shear moduli near 1 Pa, and that the same concentration of cross-linked actin has a modulus at least 100 times higher, fit recent theories for the viscoelasticity of long semiflexible polymers (MacKintosh et al., 1995; Maggs, 1997; Morse, 1998b). In networks of random coil polymers, topological restraints can have rheological effects similar to those of stable cross-links. But the origin of elasticity in semiflexible polymer networks is different, and their rheological behavior is very dependent on whether the polymer filaments are cross-linked. In particular, mechanical deformation of individual filaments in uncross-linked networks will relax too quickly to affect the rheological measurements (Morse, 1998a), except at very high frequencies ($>$100 Hz). As a result, the elastic resistance of uncross-linked F-actin is predicted to be several orders of magnitude smaller than that of a corresponding network with significant cross-links. These recent advances in theory provide a molecular description of the basis of F-actin rheology and explain the large effect of cross-linkers on actin networks that are already extensively constrained by steric interactions.

Although the reversible formation of disulfide-bonded actin dimers or oligomers appears to explain the large elastic moduli measured in many samples of otherwise similarly purified actin, there are likely to be other factors influencing the formation of filament-filament cross-links that have yet to be defined. For example, the rate of formation of the disulfide bonds is variable in different preparations, and in some fractions of purified actin prepared by gel filtration it does not appear to occur over a time course sufficient to cause large rheological changes in other samples. It is plausible that the variable rate of oxidation and the fact that it cannot be prevented by 200 μM DTT may be due to slight contamination of metal ions such as copper, an ion that both binds very tightly to G-actin and catalyzes the formation of Cys-Cys bonds (Lehrer et al., 1972; Drabikowski et al., 1977). Other contaminants, including perhaps other metal ions or metal-bound actin monomers, may also promote cross-linking independently of the disulfide formation. We found an additional complication that requires further study—that some actin solutions further purified by chromatography exhibited high elastic moduli that were not reduced by the addition of 10 mM DTT.

Whatever the cross-linking species may be in various purified actin preparations, the rheological characteristics of actin gels with high moduli, as well as the present data showing DTT-sensitive direct interactions of actin filaments with each other, appear to resolve the issue of actin elasticity in favor of the conclusion that uncross-linked F-actin networks have relatively low elastic moduli (Hinner et al., 1998; Ruddies et al., 1993b; Wachsstock et al., 1993). Therefore, additional actin filament cross-linking proteins such as ABP-280/filamin (Cunningham et al., 1992; Janmey et al., 1990), α-actinin (Wachsstock et al., 1993; Xu et al., 1998b), and possibly Arp2/3 complex (Mullins et al., 1998a,b) are essential for producing the elasticity of the actin cortex in vivo.

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