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Viscoelasticity of F-Actin and F-Actin/Gelsolin Complexes†

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ABSTRACT: Actin is the major protein of eukaryote peripheral cytoplasm where its mechanical effects could determine cell shape and motility. The mechanical properties of purified F-actin, whether it is a viscoelastic fluid or an elastic solid, have been a subject of controversy. Mainstream polymer theory predicts that filaments as long as those found in purified F-actin are so interpenetrated as to appear immobile in measurements over a reasonable time with available instrumentation and that the fluidity of F-actin could only be manifest if the filaments were shortened. We show that the static and dynamic elastic moduli below a critical degree of shear strain are much higher than previously reported, consistent with extreme interpenetration, but that higher strain or treatment with very low concentrations of the F-actin severing protein gelsolin greatly diminish the moduli and cause F-actin to exhibit rheologic behavior expected for independent semidilute rods, and defined by the dimensions of the filaments, including shear rate independent viscosity below a critical shear rate. The findings show that shortening of actin filaments sufficiently to permit reasonable measurements brings out their viscoelastic fluid properties. Since gelsolin shortens F-actin, it is likely that the effect of high strain is also to fragment a population of long actin filaments. We confirmed recent findings that the viscosity of F-actin is inversely proportional to the shear rate, consistent with an indeterminate fluid, but found that gelsolin abolishes this unusual shear rate dependence, indicating that it results from filament disruption during the viscosity measurements. The viscosity of gelsolin/F-actin complexes at very low shear rates is proportional to approximately the fifth power of the filament length. Therefore, proteins that control actin filament length can powerfully regulate the rheologic behavior of cytoplasmic actin.

Actin is the most abundant protein in the peripheral cytoplasm of nucleated cells where other proteins specifically regulate its reversible assembly into linear polymers and the attachment of these filaments to each other or to the plasma membrane (Stossel et al., 1985; Pollard & Cooper, 1986). These attributes of actin have led to the belief that it is responsible for various cell movements and for the ability of cells to resist external forces and maintain their shapes. Since this proposed function of actin is mechanical, it is important to understand actin's architectural properties, and as a starting point, investigators have tried to study the mechanics of purified actin solutions in vitro. This approach, rather than defining the basic mechanical properties of actin itself, by which to understand more complex protein mixtures, has led to controversy and quite disparate views (Stossel et al., 1987; Elson, 1988).

Purified monomeric actin polymerizes in vitro to form a 10 nm diameter filament (F-actin) population with a heterodisperse length distribution, the longest strands of which may be as long as 30 μm (Lanni & Ware, 1984; Zaner & Hartwig, 1988) and which are relatively stiff along their contour (Takebayashi et al., 1977; Fujime, 1970, Egelman, 1985). A tradition of colloid science and polymer chemistry predicts that dilute monomeric actin should be a Newtonian fluid of viscosity very near that of its solvent and that F-actin exhibits

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viscoelastic behavior, elastic because of the extensive interpenetration of the long actin filaments that tends to immobilize them, but fluid at long times, on the simple assumption that the filaments are chemically independent and therefore free to diffuse translationally or, especially the shorter filaments, rotationally (Ferry, 1980; Doi & Edwards, 1986). The concentration and length of the actin filaments and the time scale of measurement following application of a stress determine the relative extents of elasticity and viscosity. Several empirical studies have provided data in general agreement with such theoretical predictions (Zaner & Stossel, 1982, 1983; Mozo-Villarias et al., 1984; Janmey et al., 1986). The theories for rodlike unattached polymers apply to stable stiff rods of defined and uniform length, whereas F-actin is semiflexible, prone to stress-induced rupture, and heterodisperse, its average length being only grossly estimable. Hence, deviations of experimental findings from theoretical predictions are not unexpected. Some investigators, however, have considered these inconsistencies sufficiently serious to have draw conclusions at variance with mainstream theories.

First, many studies have found the viscosity of F-actin to decrease with increasing shear rate (Kasai et al., 1960; Maruyama et al., 1974; Zaner & Stossel, 1982, 1983; Opperman & Jaberg, 1984; Sato et al., 1985, 1987; Buxbaum et al., 1987). Such "shear thinning" is typical for entangled synthetic polymers and reconciled by standard polymer theory as arising from the disentanglement of independent but topologically constrained chains by the shear forces (Ferry, 1980). At shear rates equal to or less than the diffusional relaxation of independent but entangled polymers, however, the viscosity should become shear rate independent as is the case for Newtonian fluids (Ferry, 1980; Doi & Edwards, 1986). Many synthetic polymers exhibit this shear rate independent "zero-shear viscosity", but F-actin's viscosity remains shear rate dependent at the lowest shear rates hitherto reliably applied, leading some to aver that F-actin's viscosity is infinite at the limit of vanishing shear rate, a property characteristic of permanently cross-linked gels. Also in support of F-actin being a solid, Buxbaum et al. (1987) recently found F-actin's viscosity inversely proportional to the shear rate rather than proportional to the −0.8 power as predicted for unattached rods (Jain & Cohen, 1981). This unusual relationship, characteristic of an "indeterminate fluid" for which applied stress does not define a given shear rate, led the investigators to conceptualize F-actin not as a homogeneous liquid but as a system of heterogeneous solid domains which slide past each other in response to sufficient stress.

Second, some fluorescence photobleaching experiments have shown that the self-diffusion of purified F-actin is so slow in a stationary solution that the filaments appear immobilized on the time scale of the measurements limited to translational diffusion faster than 2×10^{-11} cm^2/s (Tait & Frieden, 1982; Doi & Frieden, 1984). In addition, the dynamic rigidity (G') of F-actin, the elastic shear modulus under oscillating stress, in some investigations became relatively independent of the oscillation frequency (ω) as low as 0.0012 Hz, behavior expected for a solid or a stably cross-linked gel (Maruyama et al., 1974; Jen et al., 1982; Sato et al., 1985). Sato et al. (1985, 1986, 1987) even found that dilute *monomeric* actin exhibited such frequency independence and concluded that unspecified bonds between actin molecules accounted for elastic behavior or monomeric and filamentous actin.

Some of the controversial issues arise from experimental inconsistencies. Not all fluorescence photobleaching recovery studies, for example, have found F-actin to be immobilized, and photochemical cross-linking may have been responsible for nondiffusion of F-actin in the experiments cited above (Lanni & Ware, 1984). The frequency-independent G' of F-actin and especially elastic behavior of dilute monomeric actin reported by Sato et al., which is not encompassable by any curent physical theory, were not detectable in other laboratories (Zaner & Stossel, 1983; Zaner & Hartwig, 1988; Zaner et al., 1988). In other cases, however, the problem arises from different interpretations of very similar data.

Why is the resolution of these questions important? If, on the one hand, purified monomeric and F-actin are elastic solids, then it is unclear why cells invest so much synthetic energy into the production of proteins that regulate actin assembly and cross-linking. If as yet identified forces bind pure actin monomers and filaments together in physiological solutions, then effort must go into understanding the nature of these forces. If, on the other hand, the viscoelasticity of actin resembles that established experimentally for polymers of similar structure and interpretable by conventional theories, then control of actin filament length and cross-linking are sufficient and extremely important, and the many actin-modulating proteins have explicable functions. In an effort to progress in resolving these questions, we have studied the viscoelastic properties of actin and of F-actin shortened by the plasma actin-modulating protein gelsolin (Yin, 1987) using six different types of measurements: steady shear viscosity, creep under constant stress, free oscillation, forced oscillation, and static shear moduli under very low constant stress. The results of these experiments, in combination with light scattering measurements, indicate that the rheology of gelsolin–F-actin complexes approximates what is predicted for an isotropic solution of rodlike polymers and that at least some of the anomalies of pure actin's rheological behavior derive from the enormous length of actin filaments and from alterations in filament structure that rheological measurements can induce.

Experimental Procedures

Proteins. Actin was purified from rabbit skeletal muscle as previously described. In some cases, further purification by gel filtration over a Sephadex G-150 column was also performed. Since this procedure dilutes the actin solutions, column-purified actin was only used for experiments employing a low actin concentration (Figures 3 and 6–8). Gel filtration removes actin oligomers, and traces of actin binding proteins. Since some of these contaminants have been shown to bind the fast-exchanging ends of actin filaments (Cassela & Lin, 1986) and thereby shorten them (Stossel et al., 1985), the major effect of this purification is the formation of longer actin filaments with higher shear moduli when such actin polymerizes (Zaner & Hartwig, 1988). A fraction of the actin was labeled with pyrene iodoacetamide (Kouyama & Mihashi, 1981), and the rate and extent of actin polymerization were determined by fluorescence changes as described elsewhere (Janmey & Stossel, 1986). Gelsolin was purified from human plasma by the method of Chaponnier et al. (1986). Gelsolin–F-actin complexes of defined length were prepared by polymerizing actin in solutions containing substoichiometric amounts of gelsolin and at least micromolar Ca^{2+}, conditions which previous studies have shown produce a population of filaments whose number-average degree of polymerization is equal to the actin:gelsolin molar ratio (Janmey et al., 1986).

Steady Shear Viscosity Measurements. Shear viscosity was measured over a very wide range of strain rate on a Rheometrics RFS8500 device in parallel plate geometry operating over a range of 10^{-4}–100 s^{-1}. A 50-mm plate was used with a gap of approximately 0.6 mm. Polymerization of actin

occurred within the apparatus prior to measurements. The apparatus applies a rotation at constant angular velocity to one face of a disc-shaped sample, and the viscosity is calculated from the torque measured by a transducer attached to the other face of the sample. The strain between the parallel plates varies with the distance from the center, and the shear rates reported are the maximum rates present at the perimeter. Viscosity is calculated from

$$\eta = MK/S \quad (1)$$

where η is the steady shear viscosity, M is the measured torque, and S is the maximum shear rate (at the edge of the plate), and

$$K = 2\pi/R^3$$

where R is the radius of the plate. Shear viscosity was also measured by falling ball viscometry, a method that applies a temporally constant stress to the sample which varies, however, with respect to the position relative to the surface of the sphere, and in which the shear rate cannot be independently set. A third independent measure of viscosity was also derived from creep experiments, using a torsion pendulum, as described below.

Light Scattering. Samples identical with those used for falling ball viscometry were introduced into cylindrical glass scattering tubes (200-μL sample volume). The total intensity and autocorrelation functions of the scattered light were measured at 90° with an apparatus and method described elsewhere (Janmey et al., 1986).

Shear Compliance (Creep). The strain of a sample subjected to a constant shear stress was measured by a newly constructed light weight torsion pendulum, modeled after the design of Plazek et al. (1958). A constant torque is applied to the freely suspended top face of a disc-shaped sample fixed at the bottom. Strain is calculated from the angular displacement of the top face, which is measured by means of a laser light reflected from a small mirror rigidly attached to the axis of rotation of the inertial arm suspended on the top face of the sample (Janmey et al., 1983).

Free Oscillations. The storage shear modulus, $G'(\omega)$, was measured by applying a momentary displacement to the inertial arm of the torsion pendulum and recording the resulting damped oscillatory movement. The elastic restoring force and the viscous damping are provided by the sample between the plates, with only negligible contribution from the torsion wire or air resistance to the motion of the inertial arm. In this apparatus, free oscillations are observed only if the elastic component of the material (G') is large compared to its viscous component (G''). Otherwise the system is overdamped. The frequency at which G' is measured is the resonance frequency of the system, which depends on the material properties and geometry of the sample and the moment of inertia of the pendulum. In most cases the angular frequency ω was between 1 and 10 rad/s. The storage and loss shear moduli are calculated from

$$G'(\omega) = (\omega^2 I/b)(1 + \Delta^2/4\pi^2) \quad (2)$$

where ω is the angular frequency of oscillation, I is the moment of inertia of the pendulum, $b = \pi r^4/2h$ (r is the radius of the sample, and h is its height), and $\Delta = -\ln (A_{n+1}/A_n)$ (A_n is the amplitude of the nth oscillation), and

$$G''/G' = \tan \delta = (\Delta/\pi)[1/(1 + \Delta^2/4\pi^2)] \quad (3)$$

Generally the quantity $\Delta^2/4\pi^2$ was very small, and the second term in eq 2 was taken as equal to 1.

This device is different in principle from most apparatus used to measure actin's viscoelasticity in which the amplitude and phase shift of strain relative to imposed stress in a forced oscillating system are used to calculate G' and G''.

Forced Oscillations. The storage and loss shear moduli (G' and G'', respectively) were measured by the Rheometrics device operating in the oscillatory mode. All measurements were made at an angular frequency of 10 rad/s and a maximum strain of 1%. The dynamic shear moduli were calculated from

$$G'(\omega) = K \text{ Re } [M/\theta(t)] \quad (4)$$

$$G''(\omega) = K \text{ Im } [M/\theta(t)] \quad (5)$$

where $K = 2h/\pi R^4$ (h and R are the sample height and radius, respectively), $\theta(t)$ is the angular displacement of the oscillating sample, M is the measured torque, and Re and Im are the real and imaginary parts of the functions within the brackets (Ferry, 1980; Jen et al., 1982).

Stress–Strain Measurements. The shear strain was measured at various stresses by applying a varying torque to the top face of the sample in the torsion pendulum and measuring the strain 30 s later.

Theoretical Calculations. The rotation diffusion constant of semidilute rigid rods was calculated for actin filaments with the formulation

$$D_{rot} = [\beta kT \ln (L/d)]/\eta_s L^9 c^2 \quad (6)$$

where k is Boltzman's constant, T is the absolute temperature, L is the length d is the diameter of the filaments, η_s is the solvent viscosity, c is the number concentration of filaments, and β is an empirical constant, thought to be near 10 (Zero & Pecora, 1982). Since F-actin solutions contain an exponential distribution of filament lengths, eq 6 is expected to provide only an approximation of the average diffusion constant. This equation applies for concentrations and lengths at which significant filament overlap occurs, defined by the relation

$$1/L^3 \ll c \ll 1/dL^2 \quad (7)$$

Previous results have shown that nearly all F-actin solutions that have been studied have met this criterion (Janmey et al., 1985, 1986).

Results

Dependence of Viscosity on Shear Rate. The shear viscosity of F-actin measured in both the parallel plate Rheometrics instrument and the torsion pendulum is shown in Figure 1 for shear rates varying over 7 orders of magnitude. These results are consistent with many earlier studies showing that the viscosity of actin is strongly dependent on shear rate over all values. They also confirm the striking observation of Buxbaum et al. (1987) that the slope of this plot over the same range of shear rates used by them is very close to −1.0. This shear rate dependence was observed for five different samples studied. Taking the most extreme conditions, the viscosity of F-actin at the lowest shear rate was nearly 10^7 times greater than that at the highest shear rate, in contrast to the expected viscosity of a purely viscous (Newtonian) fluid which would be constant at all shear rates. The value of the slope, moreover, is significantly greater than that predicted for isotropically distributed rigid rods in suspension. The point at the lowest shear rate attainable (4×10^{-8} s^{-1}) was derived from a creep experiment done with the torsion pendulum. The agreement of this value with that extrapolated from the Rheometrics data

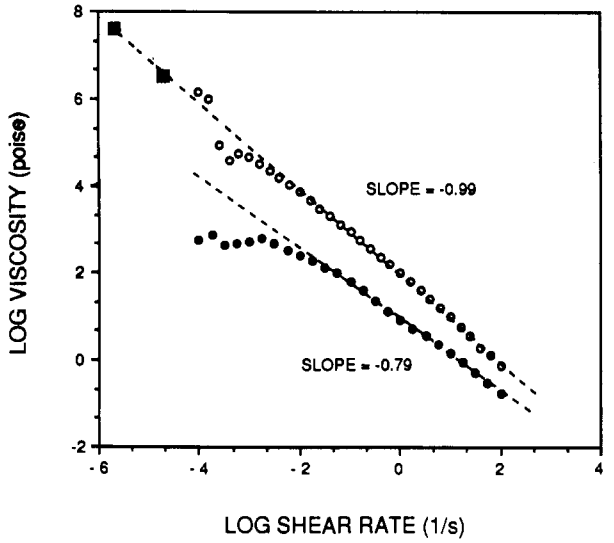

FIGURE 1: Shear rate dependence of the viscosity of F-actin in the absence and presence of gelsolin. The steady shear viscosity of 4 mg/mL F-actin in the absence (open circles) and presence (closed circles) of a 1:500 gelsolin:actin molar ratio was measured at various shear rates in a Rheometrics RMS-800 instrument at 21 °C. The polymerization of actin was initiated by addition of $MgCl_2$ and KCl to 2 mM and 150 mM, respectively, and the sample immediately transfered to the bottom plate of the rheometer. The top plate was then lowered until the liquid filled the entire gap between the plates. The first measurement was made at the lowest shear rate and begun 60 min after initiation of polymerization. Thereafter, measurements were made at successively larger shear rates. The sample was first sheared for 1 min at each shear rate during which the data were ignored and then for 3 min during which time the data were sampled and averaged. The slopes of the plots were obtained by linear least-squares fits to the data at shear rates between 0.001 and 100 s^{-1}. The squares represent viscosities determined from creep experiments using the torsion pendulum and the same preparations of proteins as were used in the Rheometrics experiments.

demonstrates that the shear rate dependence of F-actin viscosity extends to much lower shear rates than have previously been examined and confirms that consistent values for F-actin viscosity can be obtained from these two different methods and apparatus.

The nucleated assembly of actin by gelsolin in micromolar calcium produces a population of actin filaments of predictable number and length, these parameters being determined by the gelsolin concentration and the actin:gelsolin molar ratio, respectively (Janmey & Stossel, 1986; Janmey et al., 1986). Polymerization of actin with a 1:500 molar ratio of gelsolin is equivalent to breaking actin filaments by a number-average length of 1.35 μm without affecting the total polymer concentration. This treatment changes the shear rate dependence of F-actin. At the higher shear rates, the viscosity of F-actin is only slightly lowered by these low molar amounts of gelsolin, but the slope of the line relating log viscosity to log shear rate for the gelsolin-shortened sample is significantly closer to −0.8 than to −1.0. Below a critical shear rate, on the other hand, the viscosities of the gelsolin-containing samples become independent of shear rate and deviate strongly from that of pure F-actin. This transition from a strong shear rate dependence to a shear rate independent viscosity occurs approximately at shear rates equivalent to the calculated rotational diffusion constant for semidilute rodlike filaments at the concentration and length of the actin filaments employed, which is calculated from eq 6 to be 9×10^{-3} s^{-1}. Decreasing the filament length, therefore, has a large effect on the viscosity of actin at very low shear rates, has a lesser effect at high shear rates, and eliminates the indeterminate shear rate dependence of F-actin.

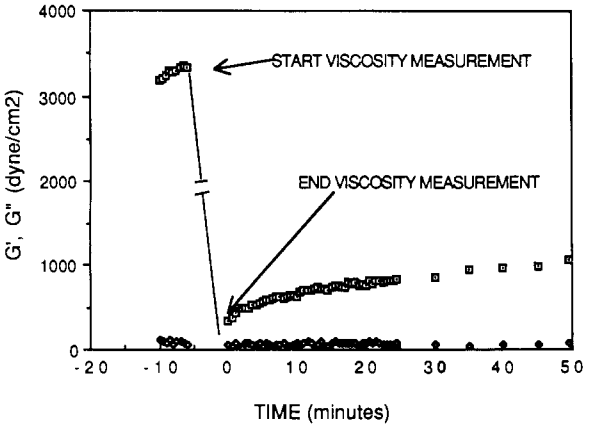

FIGURE 2: Effect of steady shear viscosity measurements on the storage and loss shear moduli of F-actin. The storage (G'; squares) and loss (G''; diamonds) shear moduli were determined from forced oscillations imposed on the samples prior to and immediately after performing the viscosity measurements shown in Figure 1. The first data point, at time 0, was taken 30 s after completion of the highest shear rate viscosity measurement shown in Figure 1. The dynamic shear moduli were determined at an angular frequency of 10 rad/s and a maximum strain of 1%.

Effect of Viscosity Measurements on the Shear Modulus of F-Actin. The storage and loss shear moduli of F-actin were measured before and after the measurement of steady shear viscosity was performed in order to determine if any structural alteration could be observed as a consequence of the large strain introduced in the sample as a consequence of the viscosity measurement. Figure 2 shows that the storage modulus G', corresponding to the sample's elasticity, is much larger than the loss modulus G'', a measure of the sample's dynamic viscosity, before application of the shear required for the steady shear viscosity measurement. A large ratio of G' to G'' is characteristic of a highly elastic material at the frequency of the measurement ($\omega = 10$ s^{-1}) in which frictional losses due to movement of the polymer molecules through the solvent are minimal, because polymer diffusion is too slow to occur during the period of oscillation. The imposed stress instead causes deformation of polymer strands that can recover when the stress is released. Figure 2 reveals that when the dynamic shear moduli are measured again, as soon as 30 s following the viscosity measurement, the storage modulus is drastically lowered, whereas G'' diminishes only slightly. As the sample is left standing, approximately 30% of the elasticity slowly recovers, suggesting that at least part of the structural alteration caused by viscosity measurements can repair when the sample is no longer strained to a high degree. Both the large decrease in G' and the increase in the G''/G' ratio are consistent with a decrease in filament length occurring during the course of the viscosity measurements.

Comparison of Light Scattering and Viscosity Changes. Figure 3A shows the progressive decrease in viscosity, as measured by rolling ball viscometry, of a series of F-actin solutions having filaments of declining average length due to polymerization with increasing molar ratios of gelsolin to actin. Since the shear rates and stresses are complex in the falling ball apparatus, a relative viscosity, defined as the ratio of the time for a ball to roll through the solution relative to the corresponding time in the solvent, is reported. At a constant average length, the viscosity increased more steeply as a function of actin filament concentration for long filaments than for short ones. At relatively high concentrations of purified F-actin, viscous flow became too slow to be measured. This apparent gelation corresponds to the nearly infinite viscosity observed repeated by other techniques for F-actin at low shear

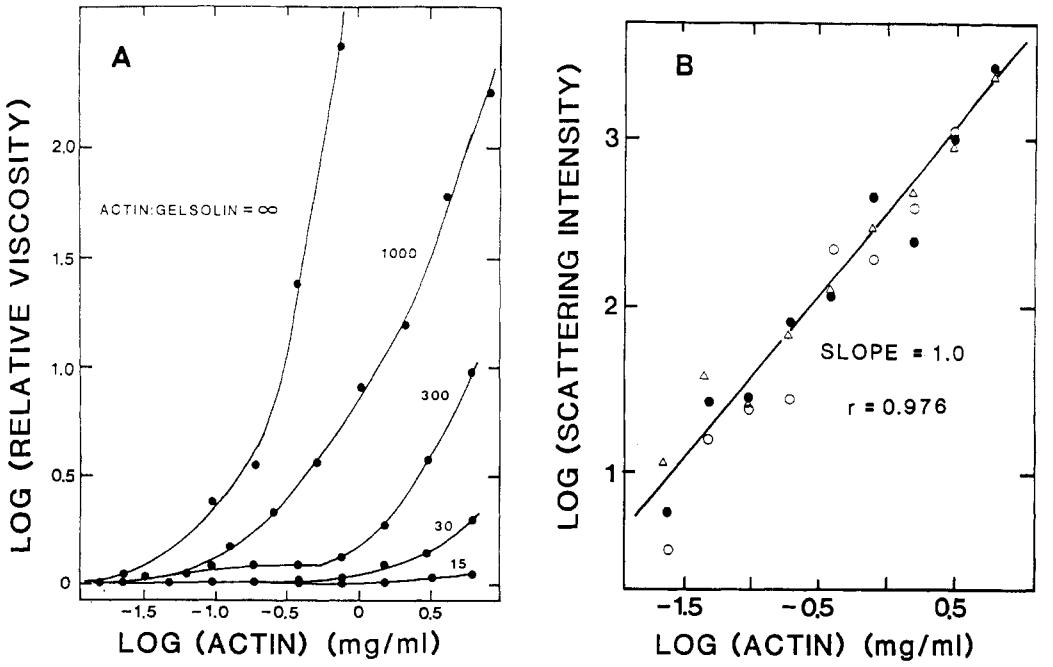

FIGURE 3: Concentration dependence of viscosity and light scattering intensity of actin filaments of various lengths formed in the presence of gelsolin. (A) Actin (6 mg/mL) was polymerized in the presence of various molar ratios of gelsolin, as shown. Two hours after polymerization was initiated, samples were diluted to the various concentrations shown and drawn up into 100-μL glass capillaries. After an additional 2-h incubation, viscosities were measured by the rolling ball method using water as a standard. (B) Part of each sample described above was added to a cylindrical glass scattering tube at the same time that the remainder was drawn into a capillary. The total scattering intensity and autocorrelation functions of the scattered light were measured at 90° with an apparatus and method described in Janmey et al. (1986). The points plotted are the average of three measurements done at different times over the entire course of the experiment. No time-dependent changes were observed for any of the samples. The symbols denoted different molar ratios of gelsolin to actin: closed circle, 0; triangle, 1:300; open circle, 1:15.

rates. Such a transition to apparently infinite viscosity is eliminated by sufficient gelsolin. These viscosity measurements, used to facilitate measurements of viscosity and light scattering in the same samples, are not strictly comparable to those of Figure 1, because the shear rate varies enormously as the viscosity of the sample changes (Zaner & Stossel, 1982).

The optical properties of the samples described above are shown in Figure 3B. The scattering intensity is directly proportional to actin concentration both in the presence and in the absence of gelsolin over the entire concentration range. These filaments are sufficiently long that the scattering intensity is not a function of length, but it is still sensitive to filament thickness. Therefore, the abrupt rise in viscosity shown in Figure 3A does not result from the formation of filament bundles or microdomains which would cause a correspondingly abrupt increase in light scattering. The autocorrelation functions derived by quasielastic scattering also showed no abrupt changes as would be expected for a concentration-dependent change in isotropy (data not shown).

Creep under Constant Shear Stress. Figure 4 shows that the increase in strain in response to a small constant shear stress depends strongly on the length of the actin filaments. In this typical experiment a relatively low concentration (0.5 mg/mL) of gel-filtered actin was polymerized in the presence and absence of a very low (1:1000) molar ratio of gelsolin. Gelsolin produces two major changes in the creep behavior of actin. First, the magnitude of the compliance is much greater at all times in the presence of gelsolin, characteristic of a large weakening of the resistance of the material to applied stress. Second, when the stress is released (at 1080 s), a much smaller fraction of the original deformation is recovered in the presence of gelsolin than in its absence, implying a loss of elasticity of the material and behavior more closely resembling that of a viscous fluid. Greater molar ratios of gelsolin resulted

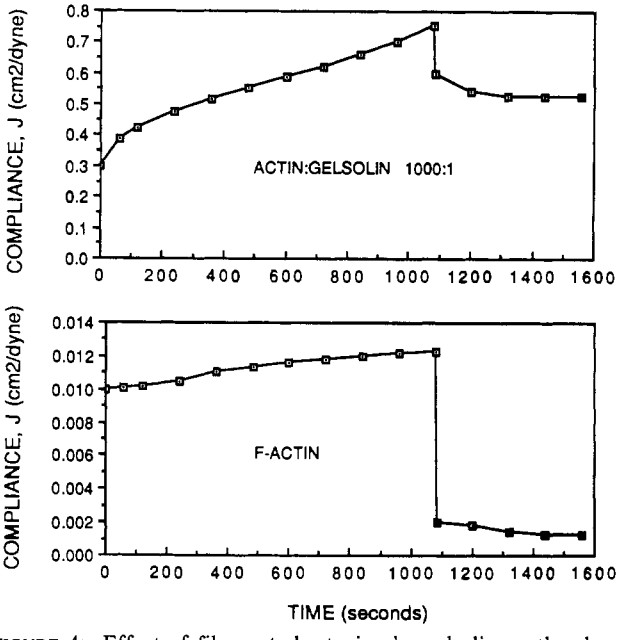

FIGURE 4: Effect of filament shortening by gelsolin on the shear compliance of F-actin. Shear compliance, the ratio of shear strain to a constant shear stress applied to the sample, is shown as a function of time for 0.5 mg/mL F-actin in the presence (upper panel) and absence (lower panel) of a 1:1000 molar ratio of gelsolin. Sufficient stress was applied in each case to produce approximately a 1% initial strain in each sample. At 1100 s, the imposed stress was reduced to zero.

in samples that flowed very rapidly in this apparatus and gave no evidence of an elastic response under these conditions (data not shown). An approximate viscosity can be calculated from these creep curves by two methods: either from the slope at long times, after the change in compliance has become constant

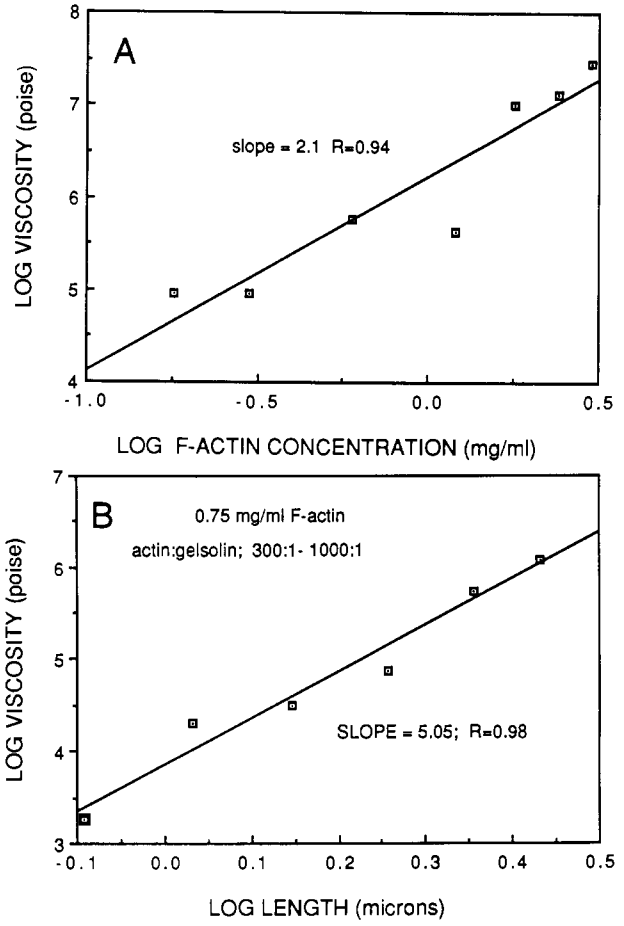

FIGURE 5: Effects of filament concentration and average length on F-actin viscosity at low shear rates. The shear viscosity was determined from creep experiments such as those shown in Figure 4, for actin samples of various concentrations (upper panel), or at a constant actin concentration of 0.75 mg/mL, for which the average length was varied by incorporation of different amounts of gelsolin (lower panel). The length reported is the number-average filament length taken as equal to the actin:gelsolin ratio times a length per actin monomer value of 1 μm/370 monomers.

with time, or from the unrecoverable compliance after a given period of stress (Ferry, 1980). The viscosity of the 1000:1 actin–gelsolin solution was 3200 and 2120 P, respectively, from the two methods, and the viscosity of F-actin alone was 1.0 $\times$ 10^6 and 0.8 $\times$ 10^6 P, respectively. The fair agreement from the two different methods of viscosity determination suggests that the sample has undergone no irreversible damage during the course of the measurement and that viscous flow, consistent with that expected for unattached rodlike molecules, is occurring. Very similar results were obtained with actin without gel filtration at similar or higher concentrations.

Concentration and Length Dependence of F-Actin Viscosity. The viscosity of F-actin at very low shear rates was measured from creep determinations over a range of concentrations. The stress, which remained constant during the course of each experiment, was chosen to give an approximately equal initial deformation ($\sim$1%) for each sample. Figure 5A shows that the low shear rate viscosity was proportional to the square of the actin concentration. The shear rates varied within a range from 3 $\times$ 10^{-6} to 5 $\times$ 10^{-5} s^{-1}.

Figure 5B shows the low shear rate viscosity of a constant concentration of F-actin polymerized in the presence of varying amounts of gelsolin in order to alter the average filament length. Under these very low shear rate conditions, the viscosity is proportional to approximately the fifth power of the filament length. A similar degree of viscosity dependence on

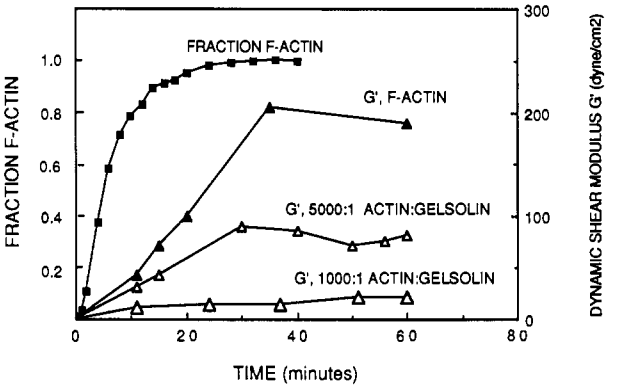

FIGURE 6: Increase in elastic modulus during polymerization of actin in the absence and presence of two different concentrations of gelsolin. Gel-filtered G-actin (0.5 mg/mL; 10% pyrene labeled) was polymerized within the torsion pendulum in the presence of various amounts of gelsolin, as shown. Occasional measurements of G' (triangles, ordinate label on right) were made by performing free oscillatory measurements. The conversion of G- to F-actin was followed in a sample not containing gelsolin by observing the increase in pyrene fluorescence (squares, ordinate label on left).

chain length has been observed for another rodlike biopolymer, schizophyllan (Enomoto et al., 1985).

Elasticity of Actin. The elastic properties of actin, evident in the recoverable compliance shown in Figure 4, were examined more closely by measuring free oscillations induced by applying a puff of air to the torsion pendulum. Figure 6 shows the time course of buildup in G', the dynamic shear modulus, as measured from the frequency of free oscillation for F-actin in the absence and presence of two concentrations of gelsolin. The appearance of elasticity is compared with the time course of actin polymerization defined as the fraction of total actin converted to F-actin as measured by changes in pyrene–actin fluorescence, a method which is sensitive to conversion of actin monomers into polymers but insensitive to filament length. The data in Figure 6 show that the onset of significant elasticity is observed only when a large fraction of actin has polymerized and that the modulus at long times is greatly reduced by low concentrations of gelsolin. Both of these observations are consistent with the idea that filament length is a powerful determinant of the elasticity of polymerized actin. Since the shear modulus of the sample determines in part the frequency of the dynamic measurement, the values of G' for the three samples are determined at somewhat different frequencies. However, more detailed measurements reveal that G' is nearly independent of ω over the entire range of average filament length and frequency shown here (S. Hvidt, unpublished experiments) and that therefore the difference in shear modulus is not an artifact of differences in the resonance frequency. Moreover, the differences in G' are reflected in differences in $1/J$ (at t = 30 s) derived from creep experiments as shown in Figure 4. Monomeric actin had no elastic behavior, exhibiting only viscous flow.

The elastic response leading to free oscillations is shown in more detail in Figure 7. After a momentary stress to the torsion arm, oscillatory motion was detected in F-actin both in the absence and in the presence of gelsolin. However, panels a and b of Figure 7 show that the frequency of oscillation (the square of which is proportional to G') was greatly diminished by gelsolin. Furthermore, the oscillations are centered around the position of the sample before application of the momentary stress to F-actin, but a significant displacement in the direction of the applied stress, due to viscous flow, is detected in the presence of gelsolin, and the oscillations are much more heavily damped in the presence of gelsolin. The damping is shown

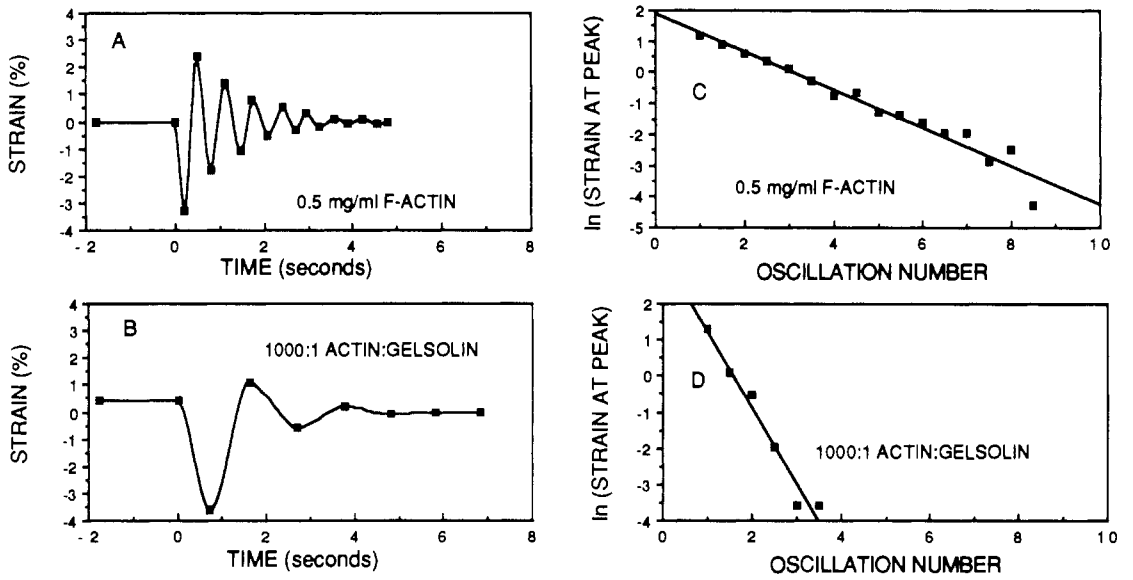

FIGURE 7: Free oscillations of F-actin with and without gelsolin. The time course of free oscillations following imposition of a momentary motion to the torsion arm is shown for two representative experiments made after 60 min of polymerization as shown in Figure 6, for F-actin without (A) and with (B) a 1:1000 molar ratio of gelsolin. The logarithmic decay in amplitude is shown in panel C (no gelsolin) and panel D (with gelsolin). The loss tangent G''/G' is calculated as described in the text.

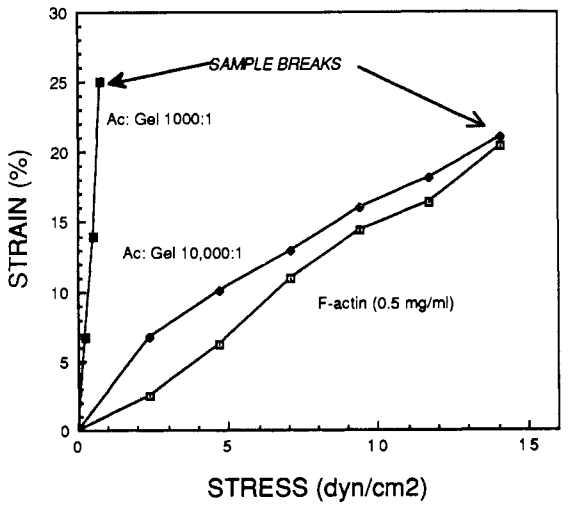

FIGURE 8: Rupture of F-actin samples and relation of stress to strain for actin with and without gelsolin. The shear strain in a torsion pendulum was measured 30 s after imposition of torsional stress for 0.5 mg/mL F-actin in the absence and presence of two molar ratios of gelsolin, as shown.

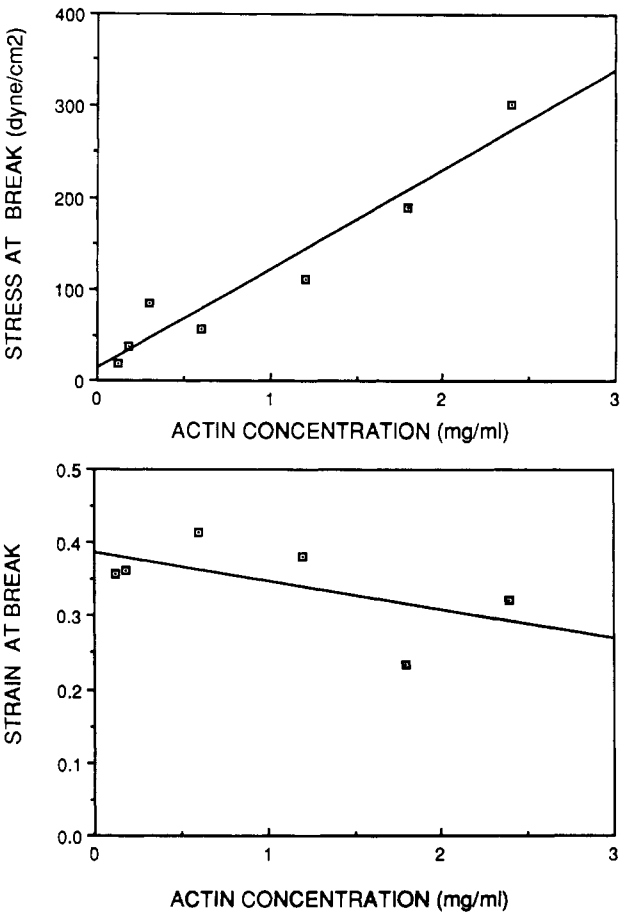

FIGURE 9: Effect of F-actin concentration on the stress and strain at which samples rupture in response to torsion. Actin samples of various concentrations were subjected to successively larger shear stresses for 30 s as shown in Figure 8. The stress and strain at which rupture occurred is shown as a function of F-actin concentration.

in detail in panels c and d of Figure 7, which show the natural logarithm of amplitude for successive oscillations. For a damped oscillator this plot should be linear, with a slope proportional to G''/G', the ratio of viscous to elastic stress. The viscous contribution is much greater for the sample containing gelsolin than for F-actin alone, suggesting that shortening of the filaments allows for more viscous flow and less elastic storage of mechanical energy by the polymer. Higher amounts of gelsolin eliminated oscillatory motion altogether, as the system became overdamped.

Abrupt Decrease in Viscoelasticity at High Stresses and Strains. The elasticity of F-actin is reflected by its ability to limit the amount of strain that a sample undergoes at a given stress. Figure 8 shows the strain of a sample as a function of applied stress for actin in the absence and presence of two different concentrations of gelsolin. As observed in the previously described experiments, a low molar ratio (1:1000) of gelsolin to actin greatly increases the strain of an F-actin sample at a given stress. Moreover, for all samples, whether containing gelsolin or not, if a critical degree of stress was exceeded, the strain increased very rapidly, and no recovery of strain occurred when the stress was removed. Such a dramatic increase in strain at a critical stress is characteristic of sample rupture. As shown in Figure 8, rupture occurred

at a much lower stress for the gelsolin-containing sample. Free oscillations could not be induced in samples that had been deformed beyond their critical stress point, consistent with irreversible damage to the sample resulting in loss of elasticity.

The stresses and strains required to break F-actin samples are shown in more detail in Figure 9, which shows the stress and strain observed at rupture for a range of F-actin concentrations. The stress needed to rupture a sample increased greatly with increasing actin concentration, but the strain leading to rupture was nearly constant. This finding is consistent with the idea that the filaments in the samples, while not being able to flow quickly enough to relieve the stress imposed, can deform elastically, but only up to some strain limit, after which they break or are otherwise altered, allowing rapid flow to take place.

Discussion

The enormous average length of filaments polymerized from purified actin in vitro (Lanni & Ware, 1984; Pollard, 1983; Zaner & Hartwig, 1988) accounts for much of the difficulty in obtaining consistent descriptions of actin's rheology independent of the method of measurement. Various theoretical calculations for rigid rods (Doi & Edwards, 1978a,b; Jain & Cohen, 1981; Zero & Pecora, 1982), from which eq 6 is derived, predict that the rotational correlation time of a 10 μm long filament in a 1 mg/mL solution is on the order of 9×10^6 s. This is the concentration and probable length of F-actin used in many studies concluding that F-actin is an elastic solid (Sato et al., 1985, 1986). Irrespective of whether any cross-links exist between overlapping filaments, such filaments cannot diffuse fast enough to accommodate to a shear rate of even 10^{-4} s^{-1}, a perturbation at or beyond the limit of most dynamic viscoelastometric apparatus. The calculations for rotational diffusion, moreover, assume an isotropic organization of the interpenetrated rods, yet both theoretical (Flory, 1956) and experimental (Ito et al., 1987) data imply that systems containing such long rods undergo phase transitions, such that isotropic and bundle domains coexist, further complicating the rheological results. Therefore, it is unreasonable to expect that the question of whether pure F-actin is a semidilute fluid or a viscoelastic solid is resolvable by extending measurements to lower and lower frequencies, and it is equally unreasonable to discount the value of studies that do not make very low frequency determinations (Elson, 1988).

Why, if the foregoing is true, have some investigators found fluid behavior for F-actin? Forcing filaments as long as 10 μm to deform at a shear rate much greater than the reciprocal of their rotational relaxation time means that at least some classes of filament motion cannot contribute to the relief of stress. The only way that bulk movement of the stressed system can occur, therefore, is if these long filaments rupture or bend. The measurements described in this paper, revealing that purified F-actin is on an order of 10–100-fold more rigid than previously appreciated and that this high consistency dissipates if the sample experiences more than a critical amount of strain, represent the first experimental evidence for such rupture in this context. This effect is likely responsible for the often observed phenomenon that F-actin samples are very stiff when a viscometric measurement begins but that soon thereafter the viscosity reaches a stable lower value (Maruyama et al., 1974; Buxbaum et al., 1987). The high rigidity measured for F-actin, moreover, explains why steel spheres producing a maximal stress of 130 dyn/cm^2 at the surface of the sphere equator remain suspended in solutions containing 1 mg/mL or less of purified F-actin (MacLean-Fletcher & Pollard, 1980). The fluidity and low rigidities of purified F-actin determined previously, therefore, must have reflected the behavior of mechanically or chemically ruptured actin filaments. This realization does not invalidate the principal conclusions of some of those studies, although it changes the interpretations of others.

Actin filament destruction by shear presumably has the effect of reducing the average filament length, although it may not always represent complete separation of filaments. Electron micrographs often reveal variable degrees of bending and even abrupt kinks of actin filaments which could arise from relatively minor dislocations of monomer to monomer contacts. Sufficient deformation may conceivably cause such displacements, thereby allowing movement of the sample in the direction of the applied stress. The observed partial recovery of G' after sample shearing suggests that the structural alterations produced by large strains at least partially heal in a subsequently unperturbed solution. A more complete analysis of the time course of such relaxations may distinguish between various mechanisms such as reannealing of broken filaments or re-formation of straight filaments from bent ones. In experiments involving constant flow, such as viscometric analyses, however, complete separation of broken filaments must occur. Actin filament rupture occurring as a result of sample deformation is uncontrolled and predictably contributes to interexperimental variations. Since copolymerization of actin with gelsolin fixes the number of length of filaments in a stoichiometrically predictable way (Janmey et al., 1986; Janmey & Stossel, 1986), the use of gelsolin–F-actin complexes contributes to the reproducibility of rheological measurements as well as providing a mechanism to vary the average filament length.

The fact that gelsolin, well known to shorten F-actin (Yin, 1987), alters F-actin's viscoelasticity in a way qualitatively similar to imposition of strain is empirical evidence that large strains rupture long actin filaments. That gelsolin eliminates the indeterminacy of F-actin's viscosity first observed by Buxbaum et al. (1987) indicates that purified F-actin samples are changing during viscosity measurements and that filament rupture occurring during the viscosity measurement is the basis of the indeterminate behavior. F-Actin shortened by gelsolin, moreover, displayed a viscosity shear rate slope of –0.8 and exhibited for the first time for F-actin a zero shear viscosity expected for independent semidilute polymers. Furthermore, the rotational diffusion constant calculated from the dimensions of independent rods predicted the shear rate at which the viscosity became shear rate independent, and the proportionality of F-actin's viscosity to at least the fifth power of length in the low shear rate regime is also consistent with behavior for stiff rods (Enomoto et al., 1985). Gelsolin-induced shortening of F-actin has previously caused it to behave in a manner predicted for independent rods in fluorescence photobleaching recovery (Doi & Frieden, 1984) and quasi-elastic light scattering experiments (Janmey et al., 1986). Removal of gelsolin or gelsolin-like molecules from actin during purification (Casella & Lin, 1986) conversely causes elongation of F-actin and increased elastic behavior. Addition of sonicated fragments of F-actin to such purified actin reduces its elasticity to a level resembling less purified actin (Zaner & Hartwig, 1988). It is impossible to rule out that mechanical shear, gelsolin, and impurities in actin, all of which shorten F-actin, decrease F-actin's elasticity by disrupting as yet uncharacterized chemical bonds between actin filaments. Since standard polymer theory adequately if not completely accounts for the effects of these perturbations on the basis of their effects on filament length, however, the burden falls on those pro-

posing the existence of such bonds to prove their existence and their importance.

Biological Implications. For practical purposes one might argue that it is biologically irrelevant whether F-actin is a viscoelastic fluid or a solid, since in either case our measurements of the stiffness of F-actin at low strains approach values determined for living cells (Chien et al., 1984; Petersen et al., 1982; Evans & Kukan, 1982; Elson, 1988). The rheology of such samples, however, is probably quite different from that of cytoplasm. The elasticity of purified F-actin derives from the length of filaments formed in vitro, but the average filament length in cells may be much shorter (Hartwig & Shevlin, 1986). As shown in this study, solutions of actin filaments shorter than those found in purified F-actin solutions are clearly viscoelastic fluids, the viscosity of which has a very steep dependence on filament length. If, for example, viscosity is proportional to the fifth power of actin filament length, as we observed, and if proteins such as gelsolin reduce the average filament length by a factor of 2, the viscosity falls to 0.03 of its original value. Hence, the many cytoplasmic proteins affecting actin filament length assume great importance for cellular rheology. The rigidity of the cell cortex containing the shortened filaments, moreover, presumably depends on effects of cross-linking proteins such as actin-binding protein (ABP, sometimes called filamin) which link the short filaments into orthogonal networks (Niederman et al., 1983) and on other proteins such as α-actinin or villin which align actin filaments into rigid bundles. Previous (Zaner, 1986) and ongoing studies of the elasticity of F-actin cross-linked by substoichiometric concentrations of ABP indicate that such networks have much higher rigidities than equal concentrations of F-actin alone, especially at low strains (manuscript in preparation). These results also show that the elasticity of F-actin resides in the stiffness of the polymer rods and does not require some additional property derived from unspecified interactions between filaments. The dual control of filament length and filament cross-linking can tightly regulate the mechanics of actin in the living cell.

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Interaction of Rabbit Skeletal Muscle Troponin T and F-Actin at Physiological Ionic Strength†

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ABSTRACT: Troponin T has been shown to interact significantly with F-actin at 150 mM KCl by using an F-actin pelleting assay and ^{125}I-labeled proteins. While troponin T fragment T1 (residues 1–158) fails to pellet with F-actin, fragment T2 (residues 159–259) mimics the binding properties of the intact molecule. The weak competition of T2 binding to F-actin, shown by subfragments of T2, indicates that the interaction site(s) encompass(es) an extensive segment of troponin T. The extent of pelleting of troponin T (or T2) with F-actin is only marginally altered in the binary complex troponin IT (or T2), indicating that the direct interactions either of troponin T (or T2) or of troponin I, or both, with F-actin are weakened when these components are incorporated into a binary complex. The binding of troponin T (or T2) is moderately ($-Ca^{2+}$) or more extensively reduced ($+Ca^{2+}$) in the presence of troponin C. The pelleting of Tn-T seen in the presence of Tn-C ($-Ca^{2+}$) and Tn-I was further reduced when either Tn-I or Tn-C ($-Ca^{2+}$) was added, respectively, to form a fully reconstituted Tn complex. As noted by others, whole troponin shows little sensitivity to Ca^{2+} in its binding to F-actin (–tropomyosin). These and other observations, taken together with the restoration of troponin IC ($\pm Ca^{2+}$) binding to F-actin by troponin T, implicate a role for the interaction of troponin T and F-actin in the thin filament assembly.

The regulation of striated muscle contraction and relaxation is largely controlled through the effects of calcium concentration on the interactions of the thin filament proteins F-actin, tropomyosin (TM),[1] and the three members of the troponin complex, troponins C, I, and T (Tn-C, Tn-I, and Tn-T, respectively). Numerous investigations have demonstrated a multiplicity of interactions between the various components and the modulation of the strengths of these interactions by the binding of Ca^{2+} to Tn-C [for recent reviews and a summary of present knowledge, see Leavis and Gergely (1984), Heeley et al. (1987), and Kay et al. (1987)]. Pertinent to the present investigation and to the molecular mechanism by which this control is exerted are the structural and interactive properties of Tn-T. This protein, now believed to be a highly asymmetric molecule, has been shown to bind to TM through two regions of interaction separated by a distance of 15–20 nm on the thin filament assembly (Ohtsuki, 1975, 1979; Mak & Smillie, 1981; Pato et al., 1981; Pearlstone & Smillie, 1981, 1982, 1983; Flicker et al., 1982; Byers & Kay, 1983; Brisson et al., 1986; White et al., 1987). One of these attachment sites involves an interaction between the T2 region (residues 159–259) of Tn-T and a segment of TM in the central region of its two-stranded coil-coil structure (Ohtsuki, 1975, 1979; Stewart & McLachlan, 1976; Chong & Hodges, 1981; Morris & Lehrer, 1984). This interaction of fragment T2 is sensitive to Ca^{2+} in the presence of Tn-C and -I, to which it also binds (Pearlstone & Smillie, 1978, 1980; Katamaya, 1979; Ohtsuki, 1979). The other attachment site, insensitive to Ca^{2+} except perhaps indirectly, is comprised of the NH_2-terminal portion of Tn-T (fragment T1; residues 1–158) and the head-to-tail overlap region of contiguous TM molecules (Mak et al., 1981; Pato et al., 1981; Pearlstone & Smillie, 1981, 1982, 1983; Brisson et al., 1986; Heeley et al., 1987; White et al., 1987). The interaction of Tn-T with TM at these loci, together with the additional connections of Tn-T with Tn-I and -C through its T2 region as well as the direct binding of Tn-I to F-actin and TM (Potter & Gergely, 1974; Hitchcock, 1975; Pearlstone & Smillie, 1983), is believed to be responsible for the fixing of the troponin complex on the thin filament assembly.

Attempts to demonstrate the existence of an additional link between troponin and the thin filament, via troponin T and F-actin, have been hampered by the poor solubility properties of Tn-T at normal physiological ionic strengths. Working at high ionic strengths (400 mM KCl) to overcome this problem, and using an F-actin pelleting assay, Potter and Gergely (1974) concluded that Tn-T interacted only weakly with F-actin if at all. In another investigation, Johnson and Stockmal (1980) concluded that Tn-T interacted directly with F-actin. This

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[1] Abbreviations: TM, tropomyosin; Tn-C, troponin C; Tn-I, troponin I; Tn-T, troponin T; SDS, sodium dodecyl sulfate; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid.

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