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Physics of Actin Networks. I. Rheology of Semi-Dilute F-Actin

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ABSTRACT The mechanical properties of cytoplasm are considered to be of underlying importance in the mechanism of cell movement and are to a large extent determined by an actin-containing cytoskeleton. Several laboratories have begun to accumulate data on the mechanical or rheologic properties of protein systems derived from the actin cytoskeleton. The focus of this manuscript is to attempt to reproduce the experimentally determined mechanical properties of non-cross-linked F-actin from theoretical considerations. It was found that a mechanical spectrum for 1 mg/ml F-actin could be calculated, which approximated experimental data, from a relaxation spectrum consisting of a long range rotational diffusion motion and short range bending motion, assuming an exponential distribution of filament lengths with a weight average length of 4 μ. The calculated spectrum underestimated the dynamic moduli at high frequencies, suggesting that a more complex actin structure is present that enhances the high frequency component.

INTRODUCTION

The mechanical properties of cytoplasm are considered to be of underlying importance in the mechanism of cell movement. This idea has its origin in the well known sol-to-gel transformation, which has been observed in cytoplasmic extracts of numerous cell types (Kane, 1975). Further evidence to support this contention is provided by the fact that changes in the viscoelastic properties of cells have been observed directly in response to various environmental perturbations (Sasaki et al., 1989; Worthen et al., 1989). The viscoelastic characteristics of the cell cortex have been incorporated into theoretical formulations of cell movement by a number of workers (Dembo, 1989; DiMilla et al., 1991; Ward and Hammer, 1993).

The mechanical properties of cytoplasm are to a large part determined by an actin-containing cytoskeleton (Stossel, 1984). The actin filaments are believed to be cross-linked into a network by a class of proteins referred to as actin gelation proteins. Among the most well studied are actin binding protein or ABP, a 540 kDa protein that has been isolated from numerous cell types and referred to as filamin by some workers, and α-actinin, a 220 kDa, calcium-sensitive protein that has also been isolated from numerous cell types (Stossel et al., 1985). The in vivo network is believed to be solated by one of a variety of proteins, in different cell types, e.g., gelsolin, severin, or actophorin (Pollard and Cooper, 1986). These proteins have been shown to shorten actin filaments by either cutting or capping them in vitro, suggesting that they can alter the mechanical properties of the network in vivo.

To elucidate the relationship between the cytoskeletal proteins, their mechanical properties and in vivo processes, several laboratories have begun to accumulate data on the mechanical or rheologic properties of actin (Kasai et al., 1960; Maruyama et al., 1974; Jen et al., 1982; Zaner and Stossel, 1983; Oppermann and Jaberg, 1985; Sato et al., 1985; Pollard et al., 1992; Ziemann et al., 1994) and actin in the presence of proteins, derived from the cytoskeleton, which can either modulate filament length (Janmey et al., 1988; Zaner and Hartwig, 1988; Muller et al., 1991) or cause actin gelation (Zaner, 1986; Janmey et al., 1990; Ruddies et al., 1992; Wachsstock et al., 1994). In addition to the empirical value of this information, e.g., for comparison with measurements made on whole cells or incorporation into theoretical models of cellular movement, this information is of great importance in defining the physical principles that underly these properties. The latter is of great importance in generating a qualitative understanding of actin networks in the cell and also can lead to simplified theoretical representations of the system, which would have important predictive value.

A difficulty in the interpretation of rheologic data for actin-containing systems is that rheologic parameters such as viscosity and rigidity change for different degrees of distortion, rate of distortion, applied force and time. The molecular cause of these properties lies in the fact that actin filaments are very stiff and, therefore, highly extended with lengths greater than 10 μ (Kawamura and Maruyama, 1970; Lanni and Ware, 1984), which leads to their interference with each others' ability to rotate freely at very low mass concentrations of protein. The concentration region over which this geometrical fact exists is called the semi-dilute regime (DeGennes, 1979). There have been significant contributions to the physical understanding of the behavior of semi-dilute systems recently (Doi and Edwards, 1986), although questions remain. To begin to delineate the physics of actin networks, the mechanical properties of non-cross-linked F-actin have been calculated from theoretical considerations.

MATERIALS AND METHODS

Actin was purified from an acetone powder of rabbit skeletal muscle by previously described methods (Spudich and Watt, 1971). The dynamic storage and loss modulus of rigidity in simple shear, $G'(\omega)$ and $G''(\omega)$ and the

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compliance, $J(t)$, were measured as described previously (Zaner and Hartwig, 1988). The protein was diluted to 1 mg/ml in buffer G (3 mM Imidazole, 0.75 mM *b*-mercaptoethanol, 0.1 mM $CaCl_2$, 0.5 mM ATP, pH 7.5), the salt concentration was adjusted to 0.1 M KCl and 2 mM $MgCl_2$, and the solution was immediately placed in the measuring device. The solution was allowed to polymerize undisturbed for 2.5 h, at which point a mechanical spectrum was measured, followed by the compliance. The compliance data were transferred to a Macintosh IIfx computer, fit to a fourth-order orthogonal polynomial, and converted to the frequency-dependent dynamic rigidities, G' and G'' (Schwarzl and Struik, 1967; Zaner and Hartwig, 1988). The strain was maintained below 10%. To determine whether these measurements disrupted the actin filaments, an additional dynamic measurement at 0.03 Hz was performed between the frequency spectrum and the compliance measurement and after the compliance determination and were found to differ by less than 5% from the measurement obtained before determining the frequency spectrum.

RHEOLOGIC PARAMETERS

The parameters by which a system is characterized mechanically has undergone considerable evolution over the last half-century. In this section, some of the relevant rheologic parameters will be briefly reviewed and related to older measurables.

Mechanical systems can be broadly divided into two classes: liquids and solids. The mechanical properties of liquids are represented by their viscosity, η, which is defined by the equation $\sigma = \eta d\gamma/dt$, where γ is the strain in simple shear and σ is the stress in units of force per unit area. The strain in simple shear is a unit-less quantity defined by the expression $\Delta x/L$, where Δx is the incremental distortion of the sample and is perpendicular to L, the initial dimension of the sample. If the fluid exhibits Newtonian behavior, η is independent of the stress and the rate of strain. Therefore, according to the above equation, a constant applied stress generates a constant rate of shear or flow with the constant of proportionality being the viscosity. In contrast, a solid obeys a quite different equation: $\sigma = G\gamma$, where G is the modulus of rigidity, measured in force per unit area. If G is independent of stress and strain and is a time-independent quantity, this equation has the form of Hooke's law and materials that obey this expression are referred to as Hookean.

These two simple equations are insufficient to characterize a wide range of materials such as solutions of polymers in the semi-dilute regime. For these materials, the viscosity is not constant at different stresses or rates of strain, but rather varies as some function of the rate of strain; nor is there a constant modulus of rigidity, which also depends on the chosen time scale. This variation in viscosity reflects underlying molecular motions that are more complex than that found in the simple fluids that exhibit Newtonian behavior. In addition to variable viscosities, some materials may exhibit elastic behavior under some conditions and viscous behavior under others.

To characterize these properties in a more quantitative way, the time-dependent relaxation modulus $G(t)$ has been defined, which replaces G in Hooke's law as defined above (Ferry, 1980). Operationally, it is measured by the application of a constant strain and observing the time-dependent stress required to maintain this strain. An alternative measurement is to apply the stress in an oscillatory manner at a frequency ω, and stress amplitude of σ_o, generating a response with strain amplitude γ_o and phase angle δ. From these values, a storage and loss modulus, $G'(\omega)$ and $G''(\omega)$, respectively, can be determined, given by $G'(\omega) = \sigma_o/\gamma_o \cos \delta$ and $G''(\omega) = \sigma_o/\gamma_o \sin \delta$. The values of G' and G'' over a range of frequencies is referred to as the mechanical spectrum. Because, in general, frequency- and time-dependent functions are interconvertible by means of a Fourier transformation, G' and G'' and $G(t)$ can be calculated from each other by this method (Schwarzl and Struik, 1967). Although these functions need to be known over an infinite range of time or frequency to perform the calculation exactly, the calculation can be performed to a good approximation when data are known over a finite range. The storage modulus represents the nondissipative component of the mechanical properties, and elastic or "rubber-like" behavior is suggested if over some range of frequency the spectrum is independent of frequency and greater than the loss modulus. The loss modulus represents the dissipative component and at very low frequencies becomes proportional to ω, characteristic of viscous flow.

To relate the rheologic measurables to molecular events, it is useful to relate them to the relaxation spectrum (Bird et al., 1977). The functions $G(t)$, $G'(\omega)$, and $G''(\omega)$, can be calculated from the relaxation spectrum, $H(\tau_h)$, which is a function of relaxation times, τ_h. The simplest interpretation of the relaxation time is in terms of the Maxwell model, which is a spring of rigidity G and a dashpot or shock absorber, of viscosity η, connected together in series from which $\tau_h = \eta/G$. The spectrum then is an array of Maxwell elements connected together in parallel. In essence this approach approximates the behavior of a non-Newtonian material by a set of Newtonian and Hookean elements. A small number of these elements often accurately specify the empiric behavior of cellular systems (Sung et al., 1988; Tran-Son-Tay et al., 1991). However, in principle, these parameters can also be related to molecular mechanisms of elasticity and viscosity or, alternatively, the molecular properties can be related to parameters with identical mathematical behavior to the relaxation time but without the limitations of the spring and dashpot formalism.

GEOMETRIC CONSIDERATIONS

A property of F-actin that has been perplexing to biochemists is the fact that at high concentrations, F-actin solutions can appear solid or gel-like when undisturbed but can be easily disrupted and then appear to be liquid. Moreover, viscosity measurements have shown F-actin solutions to be non-Newtonian with very striking shear rate dependence (Buxbaum et al., 1987). This type of behavior is often observed in polymer systems and is considered to be a reflection of the fact that the molecules are no longer entirely separate but, rather, interact with each other by either entanglements or topologically constraining each others' movements. The range of concentration over which this behavior is observed is referred to as the semi-dilute regime. Although such behavior is observed in polymer solutions, these properties are observed for F-actin at weight fractions that are 10- to 100-fold less than for synthetic polymers. The main reason for this is that F-actin is an extremely long and stiff filament that intrudes easily into the geometric domain of its neighbor.

When the polymer molecules are very far apart, e.g., an average distance between them of 10 diameters or more, they can be considered to be noninteracting and transport properties such as viscosity are determined by adding up the contributions of the individual molecules. As the solution becomes more concentrated, the motions of one particle have an ever greater influence on the motion of its neighbors. However, at some concentration the domains of the molecules, which can be defined as the spherical region traced out by the rotations of the molecule, overlap. This concentration is referred to as c^*, the polymer concentration at which semi-dilute behavior begins and depends on the filament length (Doi and Edwards, 1986). This is also the concentration at which interesting things happen to the rheologic behavior of polymer systems.

Although in reality the onset of semi-dilute behavior may be gradual, it is of value to calculate c^* for F-actin solutions. For an actin concentration of c in mg/ml, there are $c_n = 1.4 \times 10^{19}\, c$ monomers per ml. Because measurements have shown that there are about 370 monomers per μ in an actin filament (Hanson and Lowy, 1963), the total length of actin, in cm, in filaments in a solution, L_t, is $3.79 \times 10^9\, c$. For filaments of uniform length, L, the total number of filaments present, $c_f = 3.79 \times 10^9\, c/L$. The total spherical volume occupied by the filaments, V_t, then is simply $(\pi/3)\,(L/2)^3\, c_f$ or substituting, $V_t = 1.98 \times 10^9\, cL^2$. If the value of c is fixed, the filament length required for semi-dilute behavior, L^*, can be calculated by setting V_t equal to unity. For an F-actin concentration of 1 mg/ml the filament length, L^*, is 225 nm, i.e., semi-dilute behavior should be observed for filaments longer than 225 nm (84 monomers). From the expression for V_t, L^* decreases with $(c)^{1/2}$, whereas c^* decreases as the square of the length. The critical length is far shorter than the estimates of the filament length found in vitro, implying that solutions of F-actin, in the absence of large amounts of a filament shortening agent, should exhibit behavior characteristic of semi-dilute filaments.

The assumption that the filaments are all of the same length is most likely an over-simplification, and a more realistic calculation should take into account the distribution of filament lengths. The precise filament length distribution of F-actin in vitro is unresolved, although experimental data and theoretical kinetic arguments suggest that initially the filament length distribution is Poisson and slowly evolves into an exponential distribution (Miyake and Stockmayer, 1965; Kawamura and Maruyama, 1970; Drogemeier et al., 1994). Statistical mechanical considerations suggest that it is sharper than exponential (Hill, 1980). Because the broadest distribution of this group is exponential, a repeat of the above calculation using this distribution would give an upper bound for entanglement. The number, n_i, of filaments of i monomers is given by (Flory, 1953)

$$n_i = n_o(1 - p)^2 p^{i-1}, \tag{1}$$

where n_o is the number of monomers and p is the degree of polymerization, which is the probability that any particular monomer is part of a polymer. The number average length, x_n, (in numbers of monomers) is $1/(1 - p)$ and the weight average length, x_w, is $(1 + p)/(1 - p)$. The total occupied volume is then

$$V_t = \sum_i n_i v_i, \tag{2}$$

where v_i is the volume occupied by a filament of length L_i and n_i is the number of filaments of that length. Substituting the expression for n_i into the above summation gives

$$V_t = 1.45 \times 10^{-4} C \frac{1 + 4p + p^2}{(1 - p)^2}, \tag{3}$$

where c is the concentration in mg/ml as above. By setting $V_t = 1$, we can determine that the threshold for semi-dilute behavior for 1 mg/ml actin is L_n of 90 nM and L_w of 180 nM. Therefore, the properties of an exponential distribution of filaments are close to that of a uniform length distribution if the weight average length is used. However, consider a situation where the number of filaments is fixed, as would be the case where a number of nucleating sites were suddenly created. Initially, there will be a sharp distribution of filament lengths that evolves into an exponential distribution. The consequence of this is that, under appropriate starting conditions, a dilute solution of actin filaments can evolve into a semi-dilute system solely by filament length redistribution.

Because the above calculation shows that an F-actin solution is entangled at modest filament lengths and concentrations, it is of interest to consider the possibility that the system can be approximated by a solid. For this to be the case, it would be required that there be numerous overlap points that would behave as temporary cross-links. To calculate this number, we imagine that the filaments are organized into a cubic lattice. If each cube has side a, $V_t = na^3$, where n is the total number of cubes in the lattice. There are 12 sides to a cube, but each side is shared by 4 cubes so that the total actin length is $L_t = 3an$. From these two expressions, we can calculate that $a = 2.81 \times 10^{-5}\,(V_t/c)^{1/2}$, where c is the concentration in mg/ml, V_t is the total volume, which can be set to unity, and a is in cm. For a concentration of 1 mg/ml, $a = 0.280\ \mu$. However, because each vertex represents the intersection of three filaments, the spacing between two filaments is half of this value or 0.140 μ, which is about 50 monomers. This calculation is a low estimate, because the overlap points should be further apart than 140 nm if the angular and positional constraints of a cube are relaxed. The significance of this calculation is that if the filaments are sufficiently long, there will be numerous over lap points per filament, implying elastic properties consistent with some of those observed in actin solutions. However, the number of overlap points limits the ability of cross-linkers to affect the mechanical properties of actin systems, because cross-linker molecules in excess of the number of overlaps will not be effective, which is consistent with previous data (Zaner, 1986).

RHEOLOGIC SPECTRUM FOR F-ACTIN

The purpose of this section is to calculate the mechanical spectra of 1 mg/ml actin solutions from the physics of rod-like polymers and the known physical properties of F-actin. The cornerstone of the theoretical description of semi-dilute polymer systems has been the realization that at these concentrations the polymers can no longer diffuse freely perpendicular to their length but rather are confined to move along their length or "reptate" (although this term is more appropriate for flexible polymers) (DeGennes, 1979). It is envisioned that a "test" polymer is confined to a cylindrical cage composed of the surrounding polymer molecules and that it can only rotate by an increment equal to the solid angle defined by the diameter of the cage when it has diffused a distance equal to its own length. From this simple assumption, the rotational diffusion constant, D_r, can be found to be (Doi and Edwards, 1986)

$$D_r = \beta D_{r0}(\nu L^3)^{-2}, \tag{4}$$

where β is a constant that has been calculated from a geometric argument (Teraoka and Hayakawa, 1989) and determined experimentally (Zero and Pecora, 1982) to be about 1.35×10^3, ν is the number of filaments per unit volume, and D_{r0} is the rotational diffusion constant in dilute solution

$$D_{r0} = \frac{3k_b T \ln(L/d - \gamma)}{\pi \eta_s L^3}, \tag{5}$$

where k_b is Botzmann's constant, T is the absolute temperature, η_s is the solvent viscosity, d is the diameter of the rod, and γ is a geometric factor, which is close to unity. Because $\nu \propto c/L$, from Eqs. 4 and 5, $D_r \propto c^{-2} L^{-7}$. It is assumed in this formulation that the filaments are completely rigid and of uniform length. Therefore, rotational motion is the only possible relaxation process and the relaxation time of the filaments is given by $t_r = 1/D_r$, and G' and G'' can be calculated from (Doi and Edwards, 1986)

$$G'(\omega) = \nu k_b T \frac{(\omega\tau_r)^2}{1 + (\omega\tau_r)^2} \tag{6}$$

$$G''(\omega) = \nu k_b T \frac{\omega\tau_r}{1 + (\omega\tau_r)^2}. \tag{7}$$

The mechanical spectra for F-actin at concentrations ranging from 0.25 to 16 m at 1 mg/ml, calculated from Eqs. 6 and 7, is shown in Fig. 1. It was assumed that the length distribution was uniform for these curves. The frequency range of 10^{-6} to 10^4 rad/s was chosen to demonstrate a very wide range of possible behavior, although it is much greater than has been reported experimentally for actin. The frequency dependence of G' for all of the lengths show the behavior expected for a single relaxation time, which is a frequency-independent value at frequencies higher than $\omega = 1/\tau_r$ and a ω^2 dependence at frequencies lower than $\omega = 1/\tau_r$. There is a peak in G'' at $\omega = 1/\tau_r$, and G'' is proportional to ω at lower frequencies and ω^{-1} at higher frequencies. The significance of this is that at frequencies lower than $1/\tau_r$, the system behaves as a Newtonian fluid, because $G'' > G'$ and the viscosity $\eta = \omega G''$, which is constant. At frequencies higher than $1/\tau_r$, the system is elastic because G' is frequency-independent and greater than G''. The spectra for the different lengths are qualitatively similar but displaced by about 2.1 decades per doubling of length, which reflects the fact that $D_r \propto L^{-7}$. The loss modulus in the low frequency region increases by about 1.8 decades per doubling of the length and $\eta \propto L^6$.

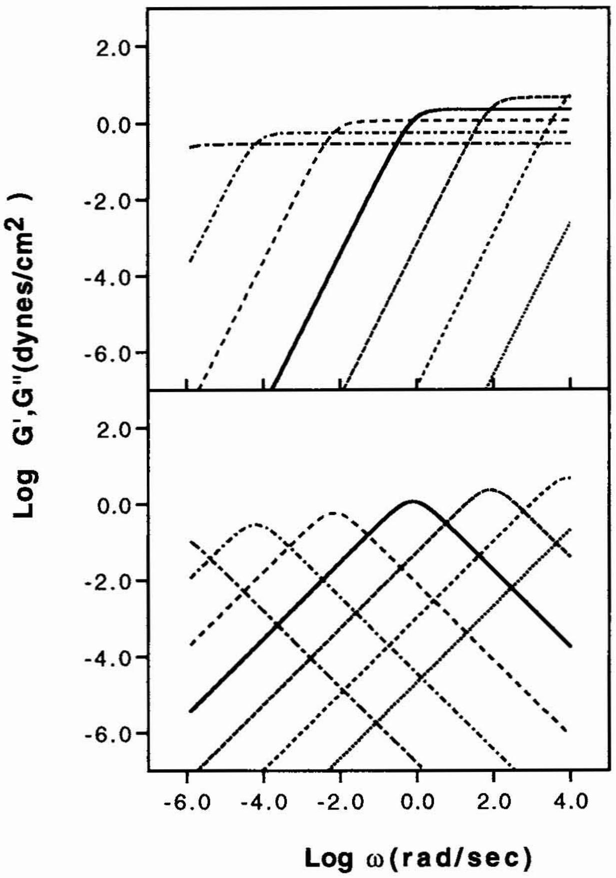

FIGURE 1 Mechanical spectra of F-actin with uniform filament length rods without bending component. (*upper* curves) G'; (*lower* curves) G''. (······) 0.025 μ; (- - - - -) 0.5 μ; (-----) 1.0 μ; (——) 2.0 μ; (– – – –) 4.0 μ; (· - - - ·) 8.0 μ; (- – – –) 16.0 μ.

Although the calculated spectra in Fig. 1 are of the same order of magnitude as some of the reported experimental data (Jen et al., 1982; Zaner and Hartwig, 1988), the peak in G'' and change in frequency dependence are much sharper than have been observed experimentally. A possible cause of this discrepancy is that the filaments in vitro are not uniform as assumed in the previous calculation but, rather, are polydisperse with a distribution that may approach exponential. To take this into account, G' and G'' were calculated incorporating the length distribution specified by Eqs. 1–3. It was assumed that the "cage" for all of the filaments was the same, i.e., each filament is immersed in approximately the same filament milieu and Eqs. 6 and 7 were replaced by a summation over the entire range of lengths. The results are shown in Fig. 2, for weight average lengths in the same range as in Fig. 1, and the shapes of the spectra now have a very different appearance. The frequency dependence of G', in contrast to Fig. 1, changes from an ω^2 proportionality to frequency independence over many decades of frequency, and there are several decades of frequency over which G' has a weak frequency dependence. In addition, the sharp peak in G'' now encompasses a much larger frequency range than is displayed in these curves. The proportionality to frequency is observed for filaments $\leq 1\ \mu$ but is lost for longer filaments, and for these shorter filaments there continues to be a 1.8 decade increase per doubling as in Fig. 1. In contrast to the uniform filament distribution shown in Fig. 2, the frequency at which Newtonian viscosity is observable is beyond the usual experimental range of frequency for filaments greater than 1 μ.

A common feature of many of the experimentally determined mechanical spectra of F-actin is that over some frequency range, G' is greater than G'' and, simultaneously, G'' increases with increasing frequency (Maruyama et al., 1974; Jen et al., 1982; Oppermann and Jaberg, 1985; Zaner and Hartwig, 1988; Janmey et al., 1990; Muller et al., 1991; Pollard et al., 1992). This behavior is not seen in the calculated spectra when either the uniform or exponential distribution of filament lengths is used. The appearance of the spectrum could be corrected mathematically if there were another relaxation mechanism in the system, with its own relaxation time, generating another peak in the spectrum. It is plausible to expect that the additional relaxation mode is due to bending motion, because an F-actin filament is not completely stiff, as assumed in the derivation of Eqs. 4 and 5, but rather is semi-flexible. The relaxation time for the flexural motions of a rod, τ_b, is given by (Oosawa and Asakura, 1975)

$$\tau_b = \zeta \frac{L^4}{\epsilon \pi^4}, \tag{8}$$

where ϵ is the Young's modulus of the actin filament, which is 1.7×10^{-17} g cm^3 s^{-2} (Oosawa and Asakura, 1975; Oosawa, 1977), and ζ is the frictional coefficient of a subunit given by

$$\zeta = \frac{3\pi\eta_s}{\ln(L/d)}, \tag{9}$$

where η_s is the solvent viscosity, which is 0.01 Poise for water. The values for the moduli were recalculated using Eqs. 6 and 7, but now including a relaxation time for bending and are shown in Fig. 3. The relaxation time for bending becomes a distribution of times when the filament length distribution is exponential. The calculation used to generate Fig. 2 was repeated by summing over all lengths but including both a rotational and bending

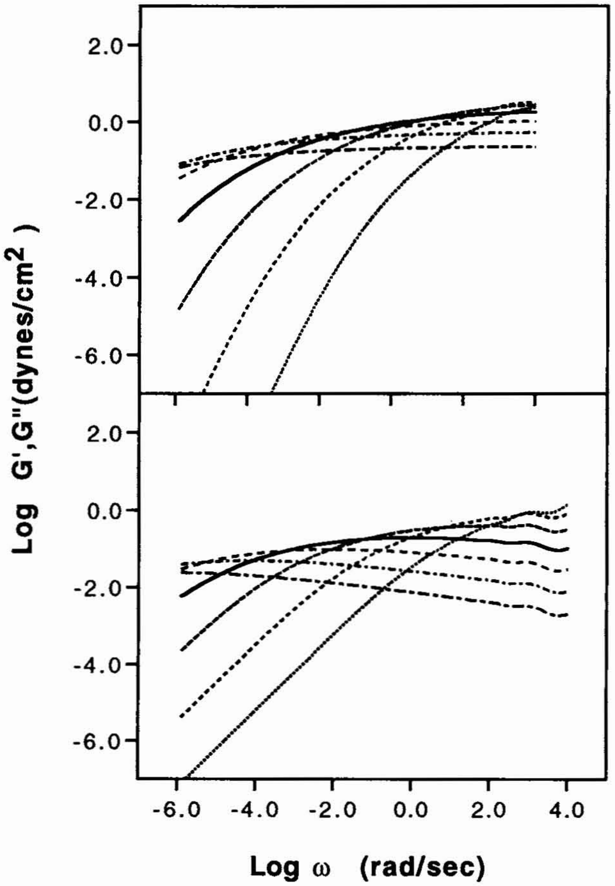

FIGURE 2 Mechanical spectra of F-actin with exponential distribution of filament lengths. (*upper* curves) G'; (*lower* curves) G''. Lengths the same as for Fig. 1.

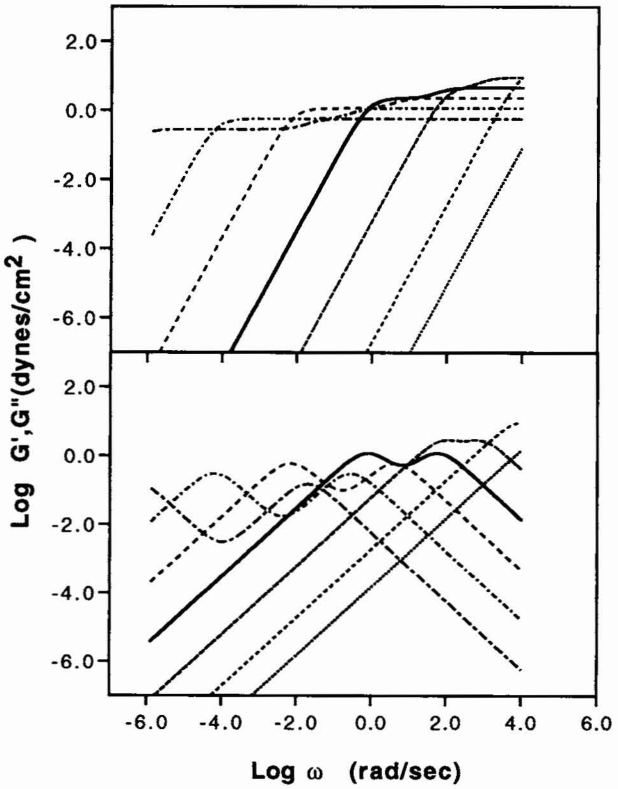

FIGURE 3 Mechanical spectra of F-actin with uniform filament length with bending component. (*upper* curves) G'; (*lower* curves) G''. Lengths the same as for Fig. 1.

component, and these curves are shown in Fig. 4. An obvious second peak appears in G'' for the uniform length filaments, which becomes increasingly separated from the rotational relaxation peak at longer filament lengths, reflecting the fact that the former depends on L^4 and the latter on L^7. The storage modulus, G', has a second plateau at high frequencies that corresponds to the new peak in G''. The appearance of the spectra at very low frequencies is unchanged, but there is now a frequency domain over which G' is frequency-independent and greater than G'', which has a relative minimum. This is characteristic of gel-like behavior, despite the fact that the system is composed of disconnected molecules. The effect of bending on polydisperse filaments is shown in Fig. 4. There is a second peak in G'', but it is much less distinct than for the uniform filaments, and the most prominent effect is that G'' is now level or increasing over most of the frequency range shown, although the frequency dependence at very low frequencies is retained. In addition, G' is frequency-dependent over more of the frequency spectrum than without the bending contribution. The frequency dependence of G' has frequently been observed for F-actin (Jen et al., 1982; Sato et al., 1985; Muller et al., 1991).

The computed spectra for 4 and 8 μ filaments with a uniform and exponential filament length distribution, with and without a bending component, are re-plotted in Figs. 5 and 6 at frequencies that approximate the experimentally accessible range. In addition, the experimental mechanical spectrum for 1 mg/ml F-actin, obtained as described in Materials and Methods, is superimposed onto the calculated data. When the filaments are uniform in length, there is a very pronounced minimum in G'', which is absent from the exponential distribution. Moreover, the differences between the spectra for 4 and 8 μ are more subtle than for the uniform distribution. Although the experimental data are of the same order of magnitude as the calculated spectra for uniform filaments, the quantitative agreement, especially for G'', is poor. On the other hand, the calculated spectra for 4 μ filaments, using an exponential distribution approximate the experimental data, especially at low frequencies.

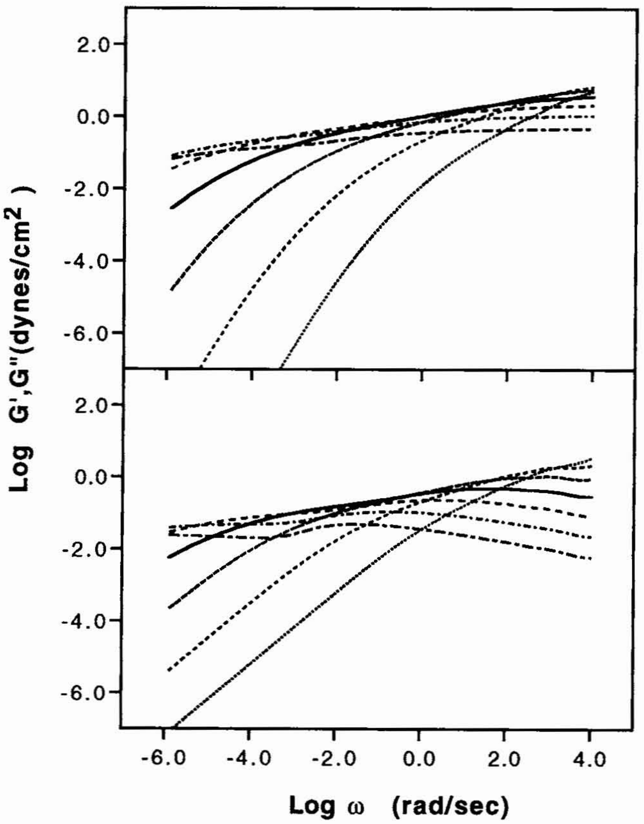

FIGURE 4 Mechanical spectra of F-actin with exponential distribution of filament lengths. (*upper* curves) G'; (*lower* curves) G''. Lengths the same as for Fig. 1.

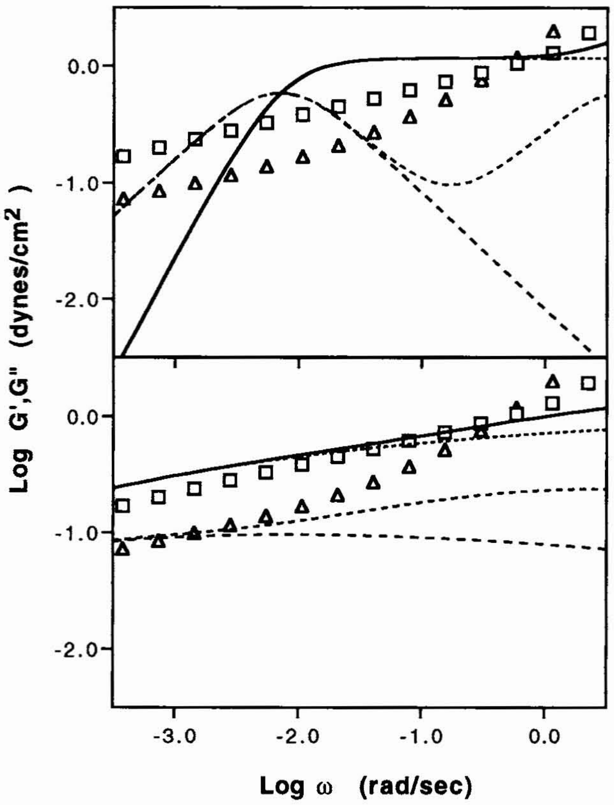

FIGURE 5 Mechanical spectra for F-actin with 4 μ filament length. (*upper* curves) Uniform filament length distribution; (*lower* curves) exponential filament length distribution. (-----) G' without bending; (——) G' with bending; (– – – –) G'' without bending; (– – –) G'' with bending; (□) G' data for 1 mg/ml F-actin; (△) G'' data for 1 mg/ml F-actin.

DISCUSSION

An understanding of the biochemical properties of actin such as the kinetics of polymerization, the control of filament length and mass, the binding of F-actin to other cellular constituents, and the mechanical properties of F-actin solutions and gels are important to elucidate cellular phenomena such as maintenance of cell structure and volume and cell motility. Although the most direct way to assess mechanical properties is by rheology, the molecular interpretation of rheologic measurements is not as well understood as other biochemical techniques presently used. In principle, if the interpretation

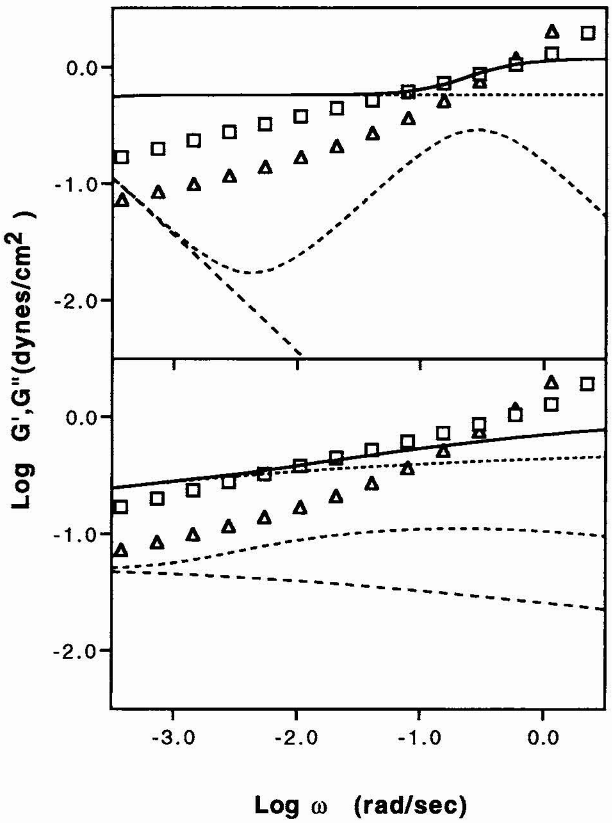

FIGURE 6 Mechanical spectra for F-actin with 8 μ filament length. Symbols are the same as for Fig. 5.

of the data were better defined, rheology of actin-containing systems could be used to study the control of length and polymerization kinetics and could complement or replace other techniques. To accomplish this goal, an attempt to begin to develop a rigorous physical model of F-actin filament solutions and gels has been performed in the present manuscript. A first step is to attempt to recreate the mechanical properties of non-cross-linked actin filaments as a function of length. This system was chosen because it is a simple system for which a large amount of data exists to compare to the theoretical predictions. The approach in the present manuscript has been to incorporate that which is known into a reasonable formulation to define the limits of our knowledge of F-actin as well as the applicability of polymer theory. The numerical simulations can reproduce a number of the rheologic properties of F-actin, although the match between theory and experiment is clearly incomplete.

There are a number of reasons for the differences between the calculated mechanical spectra and the observed mechanical spectra. Although the model of rod-like polymers in solution has had great success in the understanding of the physics of these systems, the idealizations used are not fully applicable to F-actin, which is neither completely stiff nor of uniform length distribution (Oosawa and Asakura, 1975). The uniform length assumption is particularly germane to the study of F-actin systems, because a great deal of data suggest that the filaments are polydisperse, with a length distribution that approaches exponential (Kawamura and Maruyama, 1970). This has been taken into account in the present calculation, but a more detailed analysis of this effect is certainly an important future analysis. Additionally, the bending of the filaments in solution represents an additional correction that has here been taken into account by considering the filaments as independent flexible objects. However, this approach may need to be further refined by considering the fact that filaments may be trapped between overlap points and behave effectively as much shorter filaments. Finally, neither the possibility of electrostatic interactions between the filaments, which could create temporary cross-linkages, nor the possibility of filament breakage has been taken into account, in part because the effect of these phenomena on the mechanical spectra is incompletely understood. The possibility of temporary or reversible cross-linkages has been suggested as an explanation of some of the available rheologic data for actin gels (Nossal, 1988; Wachsstock et al., 1994), and a number of models have been applied to the understanding of reversibly cross-linked synthetic polymer systems (Hong et al., 1977; Edwards, 1986).

However, a number of characteristics of the experimental spectrum can be simply reproduced by a few assumptions. Most notably, the fact that G' is greater than G'', which is often observed for actin solutions (Jen et al., 1982; Oppermann and Jaberg, 1985; Sato et al., 1985; Pollard et al., 1992; Newman et al., 1993) over a broad range of frequency, predicts the experimentally observed gel-like mechanical behavior for F-actin in this frequency range. The position of this zone, which depends strongly on the magnitude of β in Eq. 4 is accurately predicted by this model. The incorporation of an exponential length distribution into the calculation broadens the peak in G'' and the sharp break in G' so that the spectra resemble those obtained for real systems. In fact, the calculated spectra are more broad than the experimental spectra, suggesting that the length distribution of F-actin is sharper than exponential. The incorporation of a bending component changes G' from frequency-independent to frequency-dependent and causes G'' to increase rather than decrease with frequency, the magnitude of which is qualitatively similar to experimental data. Although, the magnitude of G' and G'' approximates observed values, there are some properties that are not well matched. The high frequency value of G' and G'' is significantly lower than has been observed (Jen et al., 1982) and may represent an inadequacy in the equations used or another, perhaps more complex filament interaction that is not included in the treatment presented here. In addition, there is an inverse length dependence at high frequencies that is not observed experimentally and again may represent a deficiency in the approach.

However, the similarities between experimental and calculated spectra suggest that this approach is potentially useful and begins to define future explorations.

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