Viscoelastic Properties of Vimentin Compared with Other Filamentous Biopolymer Networks

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Abstract. The cytoplasm of vertebrate cells contains three distinct filamentous biopolymers, the microtubules, microfilaments, and intermediate filaments. The basic structural elements of these three filaments are linear polymers of the proteins tubulin, actin, and vimentin or another related intermediate filament protein, respectively. The viscoelastic properties of cytoplasmic filaments are likely to be relevant to their biologic function, because their extreme length and rodlike structure dominate the rheologic behavior of cytoplasm, and changes in their structure may cause gel–sol transitions observed when cells are activated or begin to move. This paper describes parallel measurements of the viscoelasticity of tubulin, actin, and vimentin polymers. The rheologic differences among the three types of cytoplasmic polymers suggest possible specialized roles for the different classes of filaments in vivo. Actin forms networks of highest rigidity that fluidize at high strains, consistent with a role in cell motility in which stable protrusions can deform rapidly in response to controlled filament rupture. Vimentin networks, which have not previously been studied by rheologic methods, exhibit some unusual viscoelastic properties not shared by actin or tubulin. They are less rigid (have lower shear moduli) at low strain but harden at high strains and resist breakage, suggesting they maintain cell integrity. The differences between F-actin and vimentin are optimal for the formation of a composite material with a range of properties that cannot be achieved by either polymer alone. Microtubules are unlikely to contribute significantly to interphase cell rheology alone, but may help stabilize the other networks.

THREE classes of filaments, microfilaments, microtubules, and intermediate filaments, collectively termed the cytoskeleton permeate the cytoplasmic space, constitute a large fraction of total cell protein, and are believed to endow the cell with the elasticity needed to resist mechanical forces encountered in vivo (Bershadsky and Vasiliev, 1988; Elson, 1988; Schliwa, 1986). Changes in the extent or spatial pattern of polymerization of one or more of these filament types is probably essential for cell motility (Oster, 1988; Oster and Perleson, 1987), and a continuous elastic network is also proposed to be needed for some features of cell signaling (Ingber and Folkman, 1989).

Some parts of the cell contain all three filament types (Schliwa et al., 1982) and specific interactions among all three combinations of filaments have been reported (Arakawa and Frieden, 1984; Hubbard and Lazarides, 1979; Katsuma et al., 1987; Runge et al., 1981), suggesting that a single, composite network composed of interwoven microtubules, microfilaments, and intermediate filaments can exist in vivo. On the other hand, expression and polymerization of each of the three filament types are independently regulated by metabolites and specific filament-binding proteins and can be selectively perturbed by temperature changes or pharmacologic agents. Moreover, some regions of the cytoplasm appear to contain exclusively one filament type. For example, the leading edge of a motile cell contains a cross-linked actin network but little or no polymerized tubulin or vimentin, and the mitotic apparatus is rich in microtubules, but has little polymerized actin. These findings suggest that the filaments have different structural properties that can be selectively exploited to produce the material and biochemical properties appropriate to a given region of the cell. Why cells use three different types of structurally similar filaments is not known, nor are the unique features of each filament fully defined. In particular, little is known about the specific physical characteristics of each filament type and how the presence of one filament influences the formation or structure of another.

This paper reports a quantitative comparison of the viscoelastic properties of microtubules, microfilaments, and vimentin intermediate filaments, and compares them with gels composed of fibrin protofibrils, the basic structural element of blood clots, a material whose viscoelastic properties are well understood in terms of its molecular structure (Ferry, 1988). Since the gel-forming potential of biopoly-

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mers is likely to determine cytoplasmic consistency, defining the differences in the viscoelastic properties of the three biopolymers making up the cytoskeleton may be helpful in understanding how they are deployed to achieve specific structural requirements in the cell.

Materials and Methods

Protein Purification

Actin (Spudich and Watt, 1971), vimentin (Nelson and Traub, 1982) and fibrinogen (Mosher and Blout, 1973) were purified by published methods. Microtubule proteins containing 82–85% tubulin and 15–18% microtubule-associated proteins (MAPS)[1] were purified as described elsewhere (Euteneuer and McIntosh, 1981; Shelanski et al., 1973). The proteins were polymerized at concentrations of 2 mg/ml in solutions containing 2 mM $MgCl_2$, 150 mM KCl, 0.2 mM $CaCl_2$, 0.2 mM DTT, 0.5 mM ATP, 2 mM Tris pH 7.4 (actin); 100 mM Pipes, 1 mM MgCl2, 1 mM EGTA, 0.5 mM GTP, pH 6.9 (tubulin); 5 mM Tris, 1 mM EGTA, 0.1 mM DTT, 5 mM 2-mercaptoethanol, 150 mM KCl, pH 7.6 (vimentin); or 0.1 U/ml thrombin, 400 mM NaCl, 50 mM Tris pH 8.5 (fibrin). Under these conditions, polymerization is very nearly total. The weight fraction of unpolymerized protein for each case is actin: 0.2% (0.1 μM critical concentration [Stossel et al., 1985]); tubulin: 0.5% (0.01 mg/ml critical concentration in taxol [Schiff et al., 1979]); vimentin: <2.5% (0.05 mg/ml critical concentration [Steinert et al., 1981]); and fibrin: <3% (based on measured clottability [Janmey et al., 1983]).

Rheologic Measurements

Viscoelastic properties were quantified by measuring the degree of deformation (strain) in each sample subjected to a given amount of shear stress (force per unit area) for various periods of time. Most measurements used a torsion pendulum capable of applying both steady and oscillatory stresses to the samples. The apparatus and the methods are described in detail elsewhere (Janmey, 1991). Briefly, three types of measurements were employed using this instrument. In stress/strain measurements, various magnitudes of stress were rapidly imposed on the sample and the resulting deformation (strain) was measured 30 s later, (for example, Fig. 1). In dynamic measurements, the storage modulus G' was calculated from the resonance frequency of oscillations resulting from applying a momentary impulse that caused a brief strain of a few percent to the sample. G' could be measured either on a sample at rest or held by the torsion wire at a constant strain (for example, Fig. 2 and Table I). In the third type of measurement a constant stress was imposed and the slow deformation of the sample was quantified in a plot of strain vs. time (for example, Fig. 3). The frequency dependence of G' (Fig. 2, *inset*) was measured with a Rheometrics RFS8500 instrument at the Chemistry Department of Risø National Laboratory (Roskilde, Denmark). The operation of this device is described elsewhere (Janmey et al., 1988). Measurements were made at room temperature, and polymerization occurred within the sample holder of the viscoelastometer, except for the microtubule sample that was polymerized for 30 min at 37°C and stabilized with 2 μg/ml taxol to ensure that the microtubules did not depolymerize before being placed into the viscoelastometer. Taxol has been reported not to alter the viscoelasticity of microtubule networks (Sato et al., 1988).

Light Scattering

The light scattering intensity at 90° of 2 mg/ml solutions of each of the four filament types was measured using a 633-nm laser light beam and a Brookhaven Instruments (Holtsville, NY) BI2030 instrument at room temperature. Simultaneous measurements of G' were performed on separate aliquots of the same preparations.

Results

Stress/Strain Measurements

Unlike viscous liquids, which deform continuously without limit when subjected to shear stresses, viscoelastic materials attain strains which change more slowly with time and which are proportional to the deforming stress, at least at small deformations. Fig. 1 shows the strain of networks composed of equal weight concentrations of microtubules, microfilaments, intermediate filaments, and fibrin subjected to a range of shear stresses. Microtubule networks show the greatest deformability, and when the strain exceeds ~50%, the material loses its elasticity and flows without limit, like a viscous liquid. Breakage of microtubule networks at high strain was previously demonstrated by Sato et al. (1988). Vimentin networks are also easily deformable at low stresses, but unlike microtubules, they withstand large stresses and strains without losing elasticity. Rather, the more they are strained, the more resistant they become to further deformation. F-actin and fibrin networks are much more resistant to deformation (have much higher shear moduli) than either microtubules or vimentin filaments. F-actin samples differ from fibrin, however, in that, like microtubules, they rupture and begin to flow when deformed beyond ~20% strain. In contrast, fibrin, like vimentin, can be deformed to large strains under relatively high stresses and does not flow freely.

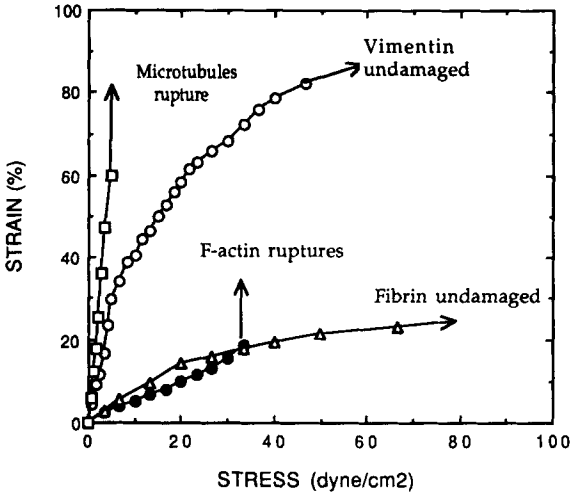

Figure 1. Stress/strain behavior of actin, tubulin, vimentin and fibrin polymers. The strain was measured 30 s after imposition of shear stress using a torsion pendulum as described elsewhere (Janmey, 1990). Solution conditions are described in Materials and Methods. The concentration of each sample was 2 mg/ml.

Strain Dependence of Dynamic Shear Modulus

The effect of strain on the viscoelastic properties of the four filament types is more readily apparent by measuring the shear modulus, the ratio of shear stress to strain, when a sample is deformed to a constant strain and then a small oscillatory deformation is applied. This quantity, defined as the differential dynamic shear modulus G', is obtained from dynamic measurements in which free oscillations of low strain are induced in the sample held at a constant larger strain. Fig. 2 shows how G' varies with strain for each of the four filament types. Microtubules exhibit a low shear modulus at all deformations and at strains >50% the sample flows freely and no longer oscillates, consistent with an abrupt decrease in elasticity. Fibrin and vimentin both exhibit strain hardening (an increase in G' at increasing strains), a feature predicted to be characteristic of polymer networks where the

1. *Abbreviation used in this paper*: MAP, microtubule-associated protein.

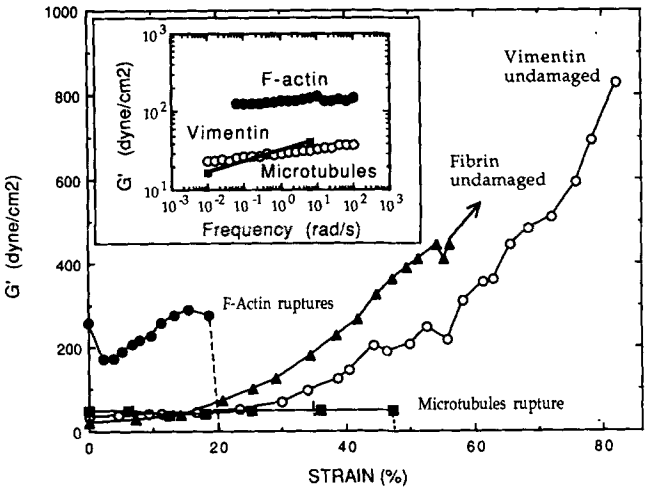

Figure 2. Strain dependence of dynamic shear modulus. The small strain modulus of the four filament networks was measured by free oscillations imposed on samples held at constant shear strains as described elsewhere (Janmey et al., 1983). The concentration of fibrin was 1 mg/ml, and that of all others was 2 mg/ml. The resonance frequency of oscillation ranged from 1 to 15 rad/s, depending on the sample volume and G'. (*Inset*) Frequency dependence of dynamic shear modulus. The dynamic storage shear moduli, G', of 2 mg/ml vimentin and 1.5 mg/ml F-actin were measured from oscillating strains of 1 or 2% using a Rheometrics instrument (Piscataway, NJ). Solution conditions were the same as those described for Fig. 1. The data on 1 mg/ml microtubules are taken from the work of Sato et al. (1988).

elastic response results from bending of the filaments (Doi and Kuzuu, 1980). The concentration of fibrin was reduced to half of that of the other polymers to compare data at high strains, because the increased resistance to deformation at high strains was larger than the static stress that could be applied by the instrument. Even when deformed to the highest strains attainable in the apparatus, fibrin and vimentin show no structural damage, as they exhibit the same shear modulus when measurements are repeated at low strains. In contrast, F-actin, though it displays the largest G' at low strains, loses the ability to undergo free oscillation when the sample is deformed to strains >20%, and after such a deformation, its shear modulus decreases by an order of magnitude when the measurement is repeated at low strain.

Frequency Dependence of Dynamic Shear Modulus

In contrast to the effects of strain, Fig. 2, *inset* shows that the shear modulus of the three cytoskeletal polymers depends very little on the frequency of measurement over a large range of frequencies from ~0.01 to 100 rad/s. This low frequency dependence over such a long range is also observed with fibrin networks (Mockros et al., 1974; Roberts et al., 1973), and implies that there are few molecular motions that dissipate mechanical energy on a time scale from 100 to 0.01 s.

Creep under Constant Shear Stress

Molecular motions that are slower than those which can be measured using oscillatory instruments, can be observed by measuring the slow deformation of samples held under constant shear stress. Fig. 3 shows the slow time-dependent increase in shear strain (creep) of samples held under a constant small stress. Experimental conditions were adjusted to produce an approximately equal initial shear strain in each sample. F-actin, like fibrin, could maintain an approximately constant strain for a long time in the presence of a relatively large shear stress. When the stress was relieved, both fibrin and F-actin recovered to very near their prestress shapes. In contrast, microtubules and vimentin filaments were highly strained by much lower stresses, and the deformation increased significantly and continuously with time. Moreover, when the stress was removed, microtubules and vimentin filaments did not recover their prestrain states, but rather exhibited unrecoverable strain characteristic of viscous flow. The slow deformation of vimentin and microtubules, which is dominated by the rate at which the longest filaments reorganize to relieve the stress, occurs at shear rates <0.0001 s^{-1}, consistent with a time constant much slower than that studied by the oscillatory measurements. Previous studies of F-actin show that the rate of creep depends very strongly on the filament length (Janmey et al., 1988).

Comparison of Filament Diameter and Elastic Modulus

The viscoelastic properties of polymers depend on polymer concentration, length, diameter, stiffness, and interactions between filaments. Table I shows that the viscoelastic differences among the four filament types shown cannot be attributed to differences in polymer concentration or filament thickness. The ionic environment of each protein was chosen to maximize the amount of polymerized material, and in each case only a negligible fraction of the protein would be expected to remain unpolymerized in these samples. The average filament length is in all cases sufficiently great compared to the wavelength of light, such that scattering intensity is approximately independent of polymer length. In this experiment, fibrin serves as a marker for filament bundling,

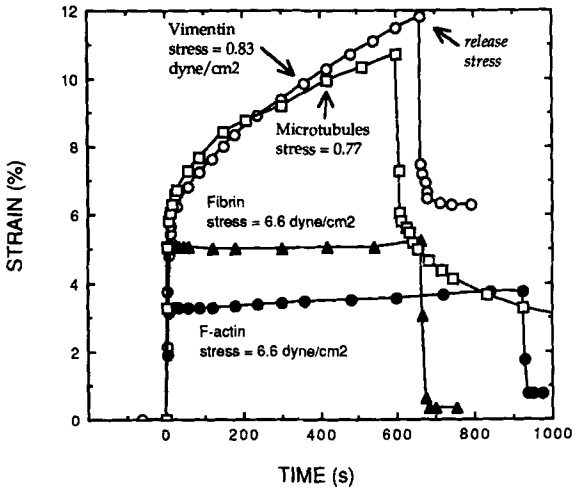

Figure 3. Slow shear flow under constant stress. The different polymer networks were strained by imposition of constant shear stresses, as noted, and allowed to recover after removing the deforming stress. The stress was imposed and released at a rate of ~1 $dyn \cdot cm^{-2} \cdot s^{-1}$.

Table I. Comparison of Light Scattering and Elastic Modulus of 2 mg/ml Actin, Tubulin, Vimentin, and Fibrin Polymers

Polymer	Scattering intensity	Shear modulus
	Arbitrary units	*Pa*
Actin	15.0	283
Fibrin	21.0	104
Vimentin	25.6	32
Microtubules	10± 4	34

Scattering of 633-nm laser light was measured at 90° using a Brookhaven Instruments B12030 instrument. The shear modulus was measured for a separate aliquot taken from the same solution of each sample. Strains imposed by the rheologic measurements ranged from 1 to 5% and frequencies from 1 to 10 rad/s.

because it has been clearly established that lateral aggregation of fibrin protofibrils depends strongly on pH and ionic strength, allowing for a continual variation in average fiber diameter over a range from fine to coarse clot conditions (Ferry, 1988; Mockros et al., 1974; Roberts et al., 1973). The ionic conditions used in this study are at the extreme of high pH and ionic strength which result in negligible lateral association of protofibrils. Table I shows that the scattering intensity correlates with the average filament diameter as estimated from electron microscopic and other data on each of the four filament types. At a constant protein concentration, the shear modulus of these biopolymers increases in reverse order to the average filament diameter.

Concentration Dependence of Shear Modulus

Fig. 4 shows how the shear modulus varies with total protein concentration for F-actin (*A*), microtubules (*B*), fibrin (*C*), and vimentin polymers (*D*). The shear moduli of both actin and fibrin are approximately proportional to the square of the protein concentration. Vimentin and microtubule networks, on the other hand, exhibit a smaller increase of G′ with increasing protein concentration. However, the differences in magnitude among the different polymer types are sufficiently large that the relative order of elastic modulus among the four proteins is maintained over the entire range of biologically relevant concentrations.

Discussion

Some basic biophysical parameters of the three major cytoskeletal fibers were determined using viscoelastometry and light scattering. Our observations reveal some similarities, but also major differences, between them. In particular, vimentin filaments, whose viscoelastic behavior has not previously been studied, possess some intriguing properties that set them apart from both actin filaments and microtubules, and these properties contribute to an understanding of their still enigmatic biological role. The rheologic properties of intermediate filaments composed of desmin or other intermediate filament proteins may differ from those formed by vimentin, and these differences may also be physiologically important.

The comparison of the viscoelasticity of F-actin, fibrin, microtubules, and vimentin filaments suggests several general features, hitherto unrecognized, that may be relevant to the function of these biopolymers in vivo. Although the properties of these polymers in vivo can be altered by other factors, these data provide a framework to understand the

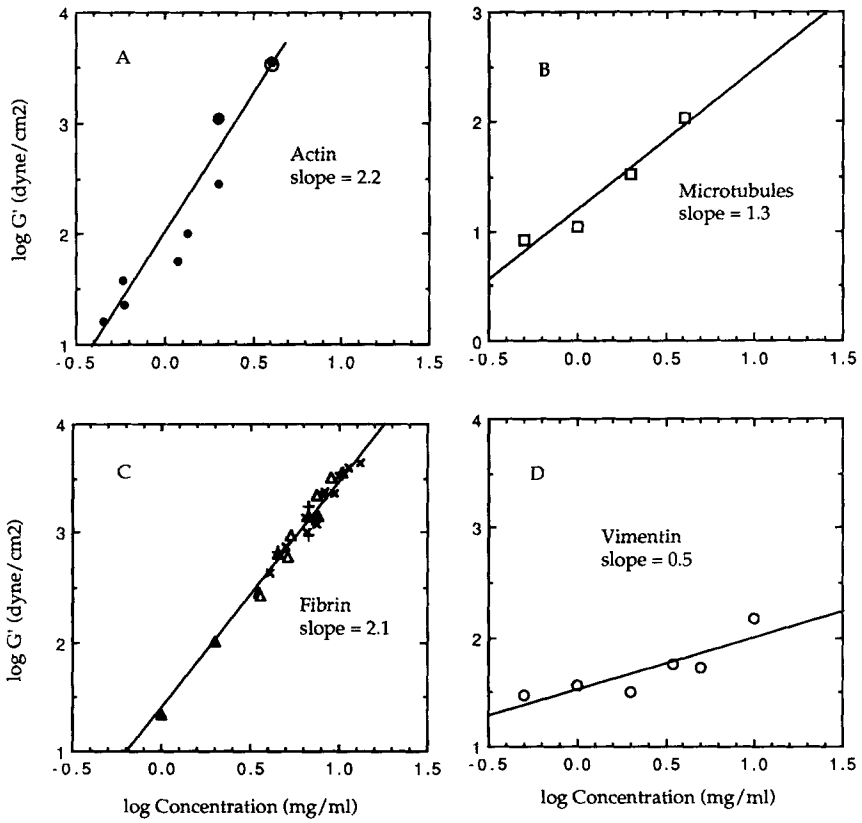

Figure 4. Concentration dependence of elastic modulus. Various concentrations of the four biopolymers were polymerized and measured as described in the legend to Fig. 1. Unless otherwise noted, all data were derived in this study using the torsion pendulum. The large open and closed circles in *A* are taken from data in Janmey et al. (1988) and Hvidt and Janmey (1990), respectively. In *C*, the + symbols are taken from Janmey et al. (1983), and Δ and x from Nelb et al. (1976, 1981), respectively, using the relation: shear compliance, $J \approx 1/G$.

basic properties of these polymers, and to predict the rheologic consequences of their remodeling by accessory proteins and metabolites. First, on a weight basis, F-actin forms networks with the greatest resistance to stresses, as long as the sample is not deformed beyond some critical strain. This makes it a logical choice for forming the network at the cell cortex where shear stresses imposed by the environment are likely to be greatest, and where the forces needed for locomotion are generated. Moreover, the rupture observed at higher strains means that F-actin networks may be rapidly fluidized when cytoplasmic flow is required. The rupture of actin networks is likely to be a property of the filaments themselves, rather than of weak interfilament interactions, since it is also observed in tightly crosslinked networks (Janmey et al., 1990). Second, intermediate filaments may serve to maintain the basic integrity of the cell. They are sufficiently flexible to allow moderate deformations without making the cytoplasm too rigid or brittle. However, under stresses that are sufficient to rupture an actin network, and which might lead to cell lysis, the strain hardening of a vimentin network would prevent excessive deformation. The differences between F-actin and vimentin are optimal for the formation of a composite material with a range of properties that cannot be achieved by a single polymer network. Third, microtubules appear to be unlikely to contribute significantly by themselves to the viscoelasticity of the cytoplasm. The large diameter of the microtubules and the relatively low concentration of cytoplasmic tubulin in the cell cortex conspire against the formation of a microtubule network of high rigidity. However, the great length and relative stiffness of microtubules may allow them to serve as stabilizing elements within a network composed of one of the other cytoskeletal fibers. For example, the microtubules observed within the interior of some lamellipodia (Rinnerthaler et al., 1988; Schliwa et al., 1982), may alter the rheology of this cortical actin network as well as facilitate organelle movement.

These results suggest which aspects of the structure of these biopolymers are most relevant to understanding their viscoelastic properties. Networks of long uncross-linked filaments that derive their elasticity from the flexing of polymer chains between points of entanglement with each other are predicted to have shear moduli proportional to a power of concentration between 2 and 2.25 (Clarke et al., 1990). Measurements of F-actin and fibrin agree well with this prediction, suggesting that although these filaments appear stiff in electron micrographs, they are sufficiently flexible over the very large distances between interfilament overlap, that some aspects of their elastic properties resemble those of flexible polymers. Flexing motions in F-actin have also been inferred from quasielastic light scattering studies (Fujime et al., 1984; Schmidt et al., 1989). When normalized by their mass/length ratios, plots of G′ versus concentration for fibrin and F-actin superimpose, strongly suggesting that the rheology of these protein networks depends on the molecular dimensions of the polymers, but not on their precise chemical structure. The prevailing theory for rigid rodlike polymers predicts a shear modulus proportional to the first power of concentration (Doi and Edwards, 1986). This prediction is more closely satisfied by microtubules (*B*), which due to their much larger diameter, are likely to be less flexible. The apparently anomalous concentration dependence of vimentin polymers suggests that the structure of these polymers may not remain constant as the concentration is varied. In particular, interpretation by theories demands that the average filament length be uniform and constant, or that the filaments be many times longer than the distance between crossover points. This latter condition is likely to be satisfied for fibrin protofibrils and microtubules, as well as for actin filaments, but the polydispersity of lengths make an exact comparison with theories difficult. How the average length of vimentin polymers depends on their concentration is not known, but the average length of the vimentin polymers in this study, determined by EM, was 3.5 μm which is slightly less than that of F-actin, but sufficiently long to ensure many crossover points per filament.

The rheology of actin, like that of microtubule networks, has been variously interpreted as deriving essentially from the liquid crystalline nature of these polymers (Buxbaum et al., 1987; Cortese and Frieden, 1988; Kerst et al., 1990), or from the steric constraints between individual polymers isotropically distributed in solution (Janmey et al., 1988; Schmidt et al., 1989; Zaner and Stossel, 1983). The comparison between F-actin and fine fibrin gels provides evidence that liquid crystal formation is not necessary for actin solutions to be viscoelastic. Fibrin gels under the conditions used in this study have been clearly shown to form isotropic networks, in which lateral aggregation of protofibrils is greatly inhibited or completely absent. At an equivalent concentrations, the shear modulus of F-actin is at least as great as that of fine fibrin gels, even though F-actin networks scatter less light. Moreover, the superposition of G′ versus concentration plots for F-actin and fibrin shown in Fig. 4 suggest that the molecular basis of the viscoelasticity of these two types of networks is similar and may be approximated by the steric hindrance of diffusion of long, interpenetrated filamentous polymers. The possibility that the other biopolymers also form viscoelastic networks independent of liquid crystal formation appears plausible.

In this model of biopolymers as primarily sterically interacting filaments, factors that alter the length or stiffness of filaments, or which introduce crosslinks between them are likely to alter the viscoelastic properties of the networks. However, specific chemical links between filaments are not necessary for a biopolymer network to be viscoelastic, nor will filament-binding proteins necessarily exert a large effect on viscoelasticity if the filament network is already sufficiently immobilized.

Our data demonstrate that viscoelastic solutions are formed by all four of the biopolymers tested under conditions where lateral filament association, or bundling, is minimal or absent. On the other hand, there is a strong thermodynamic drive for long rodlike filaments to form liquid crystalline arrays (Flory, 1956; Onsager, 1949), and ample evidence that microtubules and F-actin undergo a transition from isotropic to liquid crystalline solutions under the influence of shear stresses, other macromolecules, or even spontaneously (Hitt et al., 1990; Hou et al., 1990; Kerst et al., 1990; Suzuki et al., 1989). Such transitions are likely to modify the rheology of the solutions and may also influence cytoplasmic viscoelasticity. However, bundle formation per se does not appear to be required to explain the rheology of these biopolymer networks. Further study of the physical properties of these biopolymer networks will serve both to advance an understanding of the physiologic role of

these ubiquitous structures and as a test of theories that attempt to describe the physics of filamentous polymer networks.

We thank Victor Perez, Konrad Oexle, Gail Collins, and Jennifer Lamb for help with experiments, M. Suffness (National Institutes of Health) for the gift of taxol, and George Oster and Thomas Stossel for many helpful discussions and a reading of the manuscript. We are also grateful to Søren Hvidt and Walther Batsberg Pedersen for providing access to and assistance with their rheologic instruments.

This work was supported by U.S. Public Health Service grant AR38910 (P. A. Janmey) and National Science Foundation DCB 16070 (M. Schliwa) and by grants from NATO and the Whitaker Foundation.

Received for publication 10 August 1990 and in revised form 28 December 1990.

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