Viscoelastic Properties of F-actin, Microtubules, and Intermediate Filaments, the Major Biopolymers of the Cytoskeleton

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ABSTRACT

The rheologic properties of gels formed by the three types of cytoskeletal polymers were measured and related to the structures of the individual biopolymer filaments. The magnitude of the elastic shear modulus and the dependence of this modulus on the degree of strain are particularly different among the three polymer types. At a constant mass concentration, F-actin makes gels with a larger shear modulus than the other polymer types. This modulus is remarkably independent of the frequency of deformation, but very strongly dependent on the strain. In contrast, intermediate filaments composed of vimentin are very strongly strain-hardening, and can withstand much higher stresses compared to gels of F-actin or microtubules. The rheologic properties depend strongly on the effects of other proteins and metabolites that alter filament structures.

INTRODUCTION

The mechanical properties of most cell types, especially cells which move or undergo large structural changes are dominated by long filamentous biopolymers composed the proteins actin, tubulin, or any of several types of intermediate filament proteins[1,2]. These polymers are generally different from most synthetic polymers in that they are very long, on the order of several microns, and very stiff, with persistence lengths ranging from near one micron for intermediate filaments, to around 10 microns for F-actin[3] and to almost 1 mm for microtubules[4]. In some parts of the cell only one type of filament is found, and in others all three are interdigitated. The three chemically distinct types of filaments also have distinctly different viscoelastic properties which can be interpreted in terms of the different structures of the polymers.

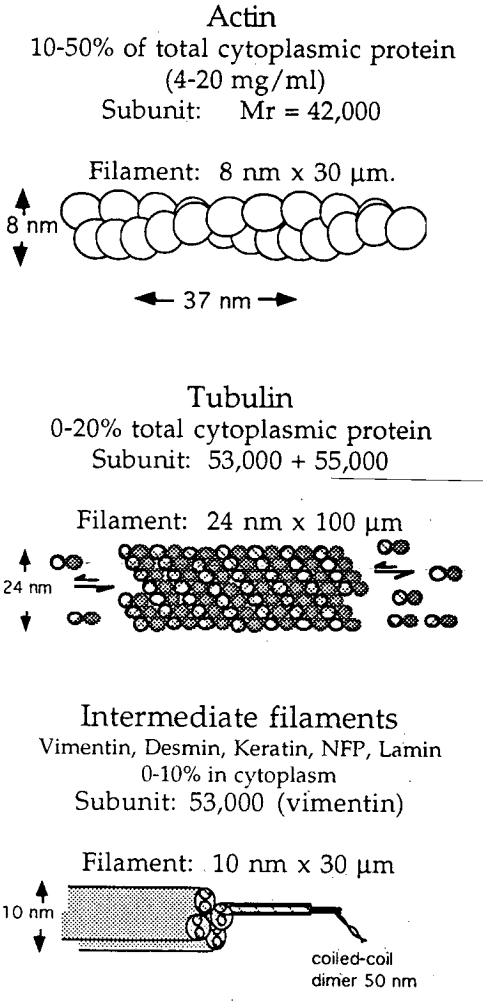

Figure 1. Schematic description of the three cytoskeletal polymers

In addition to the inherent viscoelastic properties of the pure filaments, the rheology of gels are also strongly dependent on the effects of other proteins or metabolites that alter filament structure or inter-filament associations.

In addition to their relevance to cell biology, cytoskeletal polymers are also an excellent model system to study polymer mechanics because it is possible to observe by fluorescence microscopy the diffusion of an individual polymer chain within a network.

MATERIALS AND METHODS

Actin, tubulin, and vimentin were purified as described elsewhere[5].Rheologic measurements were made in collaboration with Søren Hvidt using either a Rheometrics RFS instrument or a light weight torsion pendulum[7]. Video microscopy of rhodamine phalloidin labeled F-actin and image analysis were done as described elsewhere[3,8].

RESULTS

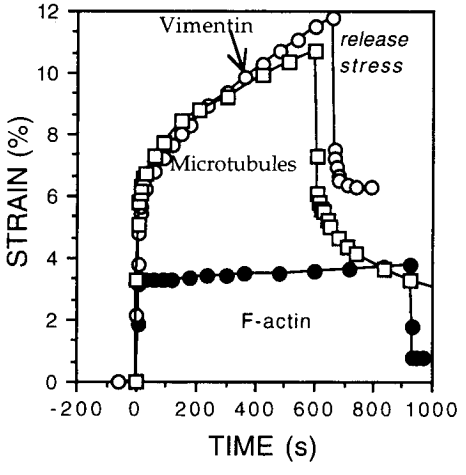

Figure 2. Creep of 2 mg/ml actin, fibrin, tubulin, or vimentin filaments

Creep under constant shear stress

When subjected to the same deforming stress, aqueous suspensions of microtubules and vimentin filaments creep much faster and show less elastic recovery after the stress is released compared to F-actin at the same concentration. These results show that the long filaments are not intrinsically crosslinked into permanent networks, but exhibit varying amounts of very slow viscous deformation and elastic recovery consistent with these biopolymers forming highly interpenetrated solutions similar those of highly entangled polymers.

Strain dependence of shear moduli.

Even though vimentin filaments creep at slow rates, at short times, or frequencies greater than 1 rad/s, they exhibit an elastic response, and a storage shear modulus that increases very strongly with increasing deformation. F-actin also exhibits such a strain-hardening, but in contrast to vimentin actin gels rupture when deformed beyond approximately 20% shear strain. Microtubule solutions have a relatively low shear modulus at low strains and rupture easily, exhibit only viscous slow when deformed beyond 20% strain.

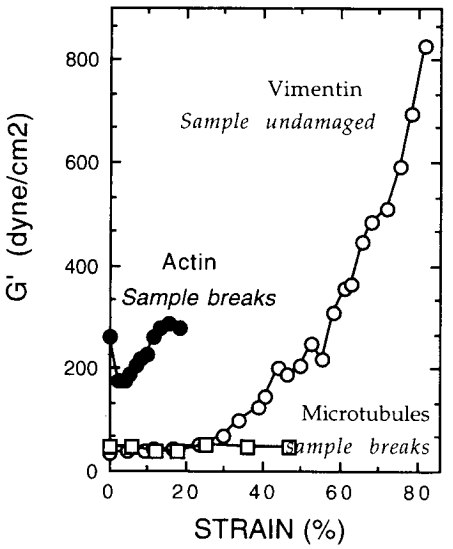

Figure 3. Strain hardening of vimentin, and rupture of actin and microtubule networks at high strains. 2 mg/ml protein.

Effects of actin-binding proteins.

In addition to basic differences between different polymer types, the rheology of a single filament system can also be altered by proteins that remodel the individual filament or which link filaments together. As one example, the storage shear modulus (G') of purified F-actin containing filaments of average length 20 microns is on the order of 200 Pa. Addition of a 1:200 molar ratio of the actin filament severing protein gelsolin, reduces the average filament length to 0.7 microns and G' to approximately 1-3 Pa. Crosslinking these short filaments with the protein ABP reforms a gel with a shear modulus as large as that of the original long filaments. Metabolic regulation of such proteins is likely to be involved in many morphologic changes that cells undergo when they are stimulated by various agents

Imaging of single polymer strands.

The very long contour length, the large bending stiffness, and the availability of specific fluorescent dyes for actin filaments and microtubules have allowed the use of high resolution fluorescence microscopy to observe single polymer strands in solution. As one example of the utility of such images, Figure 4 shows a single actin filament, labeled with rhodamine phalloidin, within a network of invisible, unlabeled actin filaments that form the surrounding environment. Measurements of the contour fluctuations of such filaments in dilute solution or within semi-dilute or entangled solutions permit estimation both of the bending stiffness of the filaments and the center-of mass diffusion of the polymers strand under minimally perturbing conditions[3,4,8].

Analysis of individual video frames taken over the course of many minutes can give information about both the filament interactions with its neighbors, and about the nature of the environment surrounding this individual polymer. By studying the motions of the ends of the filaments as well as the lateral excursions of the filament contour, such images have been used to evaluate theories of the dynamics of polymers in semi-dilute solutions and have given excellent agreement with theories for reptation within a tube .

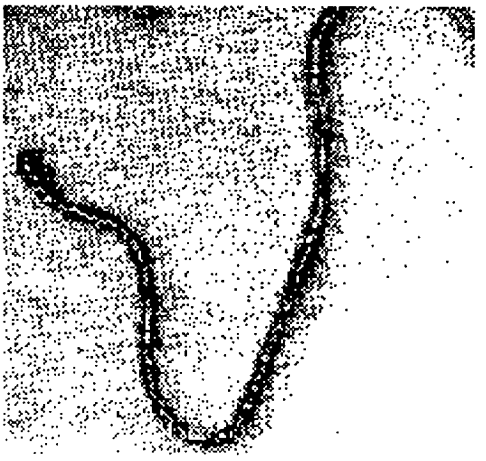

Figure 4. Fluorescence image of a single actin filament in a solution containing 2.4 μM total actin subunits in filamentous form, of which 5 nM were assembled into fluorescent filaments.

DISCUSSION

The large variety of rheologic properties exhibited by the three different types of cytoskeletal polymers is likely to be exploited by the cell to perform specialized functions in different cell types and under different conditions. The viscoelasticity measured in vitro suggests that intermediate filaments may provide a basic framework to maintain cell integrity under mechanical stresses, while the more highly variable rheologic properties of F-actin, especially when modulated by its accessory proteins suggest a more prominent role for this protein in rapid, localized changes in cell structure that occur, for example, when a cell is stimulated to move.

In addition to their interest to biology, cytoskeletal protein polymers are also an excellent experimental system to test basic concepts of polymer physics, especially as applied to stiff or semi-flexible polymers, for which a theoretic understanding is still being developed.

ACKNOWLEDGMENTS.

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