Some Perspectives on the Viscosity of Actin Filaments

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ABSTRACT   Measurements of the dynamic viscosity of various actin filament preparations under
conditions of low and controlled shear: (*a*) confirm the shear rate dependence of F-actin viscosities
and show that this dependence obeys the power law relationship observed for entangled synthetic
polymers; (*b*) permit estimation of the extent to which shear artifact amplifies changes in the apparent
viscosity of F-actin measured in a falling ball viscometer; (*c*) show that gel-filtration chromatography
of actin and the addition of cytochalasin B to F-actin bring about small (20–40%) changes in the
viscosity of the F-actin solutions. These variations are consistent with alterations in the actin-binding
protein concentrations required for incipient gelation, a parameter inversely related to average
filament length. Therefore: (*a*) changes in the viscosity of F-actin can be magnified by use of the
falling ball viscometer, and may exaggerate their biological importance; (*b*) chromatography of actin
may not be required to obtain meaningful information about the rheology of actin filaments; (*c*)
changes in actin filament length can satisfactorily explain alterations in F-actin viscosity exerted by
cytochalasin B and by chromatography, obviating the need to postulate specific interfilament
interactions.

The existence of actin filaments at the periphery of many cells
has encouraged further study of the solution properties of F-
actin. The rheologic study of F-actin solutions in the absence
or presence of modulating factors, a direct means of obtaining
information with regard to their mechanical properties, could
allow further understanding of molecular structure (1).

A miniature version of the falling ball viscometer has become
popular for measurement of the rheologic properties of actin
and other biological polymers (2–10). This device has the
advantages of requiring small quantities of material, exerting
low stress and, facilitating the measurement of many samples.
It has therefore been useful for the identification of factors that
modulate the properties of F-actin solutions and as a conven-
ient assay for these factors in their subsequent purification.
However, the rate of strain and apparent viscosity are depend-
ent on each other using a device of this kind, making quanti-
tative interpretation of data obtained from non-Newtonian
materials a formidable task. In principle, this device can ex-
aggerate differences in viscosity between samples of a material
such as F-actin, whose viscosity depends exponentially on rate
of shear, suggesting the existence of more drastic molecular
events than those taking place.

The problem caused by the fact that rate of strain is depend-
ent on apparent viscosity can be overcome by using an instru-
ment with a variable rate of strain such as the cone and plate
viscometer, couette viscometer (11), or the dynamic viscometer
which we have previously described (12). The last instrument
measures dynamic viscosity, $|\eta^*|$ by an oscillatory motion as a

function of frequency. It has been shown that the dependence
of dynamic viscosity on frequency very closely approximates
the dependence of viscosity on rate of shear, so that frequency
in units of radian/s closely approximates rate of strain in units
of sec^{-1} (13). The frequency can be varied independent of
viscosity in this instrument, enabling measurements of viscosity
over a range of frequencies.

It has been recently reported that the apparent viscosity of
actin prepared by a widely used method (14) is strikingly lower
in the falling ball viscometer than that of the same actin
subjected to an additional purification step involving gel-filtra-
tion chromatography with Sephadex G-150. The components
separated by gel-filtration responsible for the viscosity changes
are not known and are evidently present in minute amounts.
In contrast, the apparent viscosity of actin filaments prepared
from monomeric actin before and after chromatography dif-
fered by only a small amount when measured in an Ostwald-
type viscometer (6), widely used in the past to study actin
rheology. A large change in apparent viscosity in the falling
ball viscometer in contrast to a small change in apparent
viscosity in the Ostwald viscometer was also noted for actin
filament solutions exposed to the fungal metabolite cytochal-
asin B (5) and to a number of proteins isolated from cells (7,
10).

The large change in the viscosity of actin after gel filtration,
as measured in the falling ball viscometer, has the immediate
implication that further purification of actin is necessary to
obtain any meaningful experimental results. Moreover, on the

assumption that viscosity values obtained by means of the Ostwald viscometer are directly related to filament lengths, it was postulated that length changes could not account for the impressive differences in viscosity values obtained with the falling ball viscometer. Because cytochalasin B and the protein factors bind to the ends of actin filaments, it was necessary to speculate that this binding *per se* reduced discrete but unspecified interactions between filaments (5, 6). Extending this logic, these interactions have been taken to be the end-to-side contacts between filaments which are blocked by agents that bind to the ends of actin filaments (7, 15).

The data obtained by the falling ball viscometer was obtained at a constant stress, hence a variable rate of shear. The effect of rate of shear was not estimated in these studies. Since the conclusions drawn in these studies has important bearing on both the way in which actin is prepared and the molecular mechanisms responsible for the rheologic properties of F-actin solutions, we have compared rheologic measurements of various actin preparations as obtained in the falling ball viscometer and the dynamic viscometer. We will calculate the effect of rate of shear on apparent viscosity and discuss the implications of data obtained by various viscometric methods.

MATERIALS AND METHODS

Proteins

Monomeric actin (G-actin) was prepared from rabbit skeletal muscle by the method of Spudich and Watt (14). A portion of the actin prepared in this way was purified further by gel-filtration chromatography on Sephadex G-150 columns (16). Actin-binding protein (ABP) was purified from rabbit pulmonary macrophages by the method previously described (17).

Viscosity and Gel Point Determinations

Rheologic measurements were made using a previously described instrument (12). The instrument measures rheologic properties of materials by observing the motion of a mica strip through a sample. The mica strip is suspended from a rod attached to a tilt table on which the sample is placed. Motion is induced by tilting the table to a fixed angle or in an oscillatory fashion to obtain dynamic viscosity measurements. The stress applied is a pure shearing stress and was kept below 0.05 dynes/cm^2, at a frequency of between 0.02 and 0.6 radians/s. The complex dynamic viscosity, $|\eta^*|$, was calculated from the following expression:

$$|\eta^*| = (G'^2 + G''^2)^{1/2}/\omega \qquad \text{Eq. 1}$$

The complex dynamic viscosity has been shown to be approximately equal to the steady state viscosity, η, at a shear rate γ, numerically equal to the radian frequency ω, for a wide variety of synthetic polymers (19). Actin samples were diluted to a concentration of 1 mg/ml in buffer A of Spudich and Watt (14), and polymerization was initiated by the addition of KCl and MgCl$_2$ to give final concentrations of 0.1 M KCl and 2.0 mM MgCl$_2$ respectively. The samples were immediately put into the viscometer and polymerization was monitored by measuring $|\eta^*|$ at 0.2 radian/s every 15 min. Frequency-dependent measurements were begun when $|\eta^*|$ reached a constant value, usually at 2.5–3 h.

The results obtained by means of the method described above were compared with rheologic information on samples of the same actin solutions tested in a small-volume falling ball viscometer (4). Immediately after addition of salts to initiate actin assembly, samples were drawn up into 100 microliter pipettes (ID 0.05 in.) and sealed at the bottom. Measurements were made by measuring the time required for a stainless steel ball (0.025 in.) to fall ~6.5 cm, at a tube inclination of 80°. This data was converted to apparent viscosity, by calibration with 100% Glycerol at 25°C. To ascertain the effect of actin oligomers on the polymerization of chromatographed actin (F-actin), sonicated fragments of polymerized chromatographed actin were added to unpolymerized chromatographed actin, at a concentration of 0.05 mg/ml immediately before the addition of salts.

The concentration of macrophage actin-binding protein required for incipient actin filament gelation was determined as previously described (18). The weight average filament length, L$_w$ was then calculated from (21):

$$L_w = \frac{[Actin]}{2[ABP] \times 370} \qquad \text{Eq. 2}$$

where the concentration of actin and ABP are in moles/liter and the numerical

factor of 370 is the number of actin monomers per micrometer of filament length (19).

RESULTS

Fig. 1 depicts the relation between the dynamic viscosity and frequency of several actin preparations as measured by the
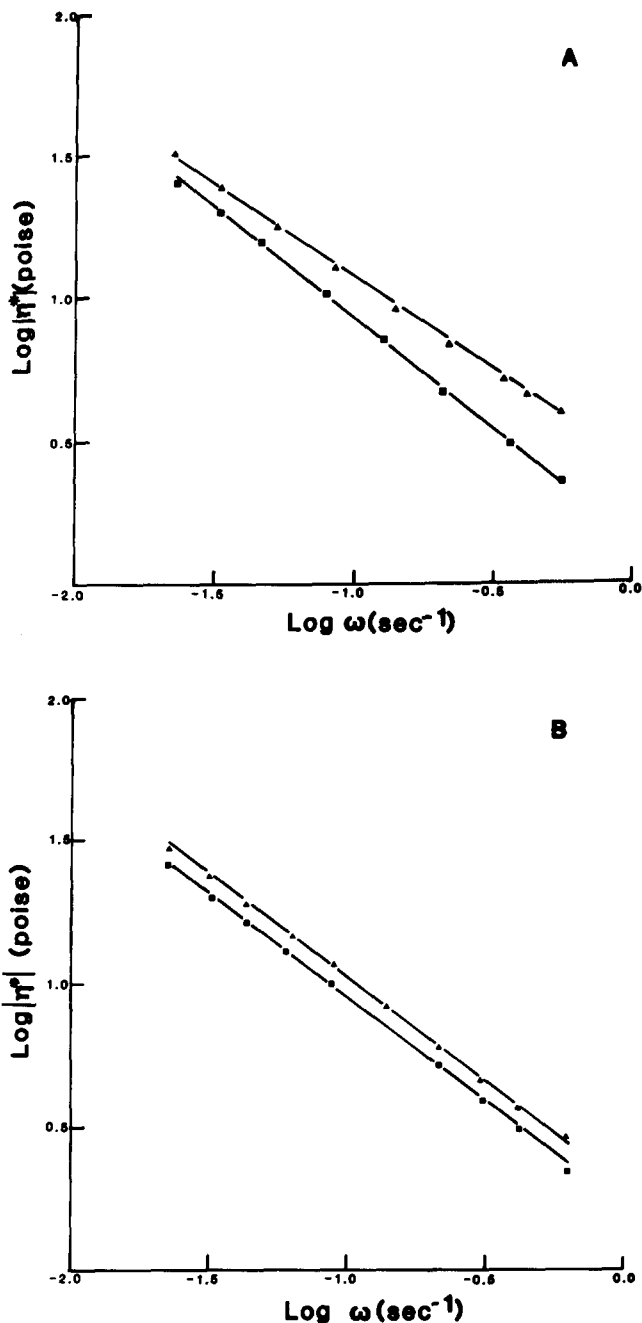

FIGURE 1 Logarithm of dynamic viscosity $|\eta^*|$ plotted against the logarithm of frequency (ω), for several preparations of F-actin. A) (■) 1 mg/ml Actin; (▲) 1 mg/ml chromatographed actin with 1/20 sonicated, chromatographed F-actin added at initiation of polymerization; B) (▲) 2 mg/ml Actin; (■) 2 mg/ml actin polymerized in the presence of 5 μM cytochalasin B. All actin samples were diluted in Buffer A (2 mM Tris, 0.2 mM ATP, 0.2 mM CaCl$_2$, pH. 7.5). Polymerization was initiated by adjusting the salt concentration to 0.1 M KCl and 2 mM MgCl$_2$. The sample was then immediately transferred into the curvette of the viscoelastometer. Sequential measurements of $|\eta^*|$ were made at 0.2 radian/sec until a constant value was reached, at which time frequency dependent measurements were begun.
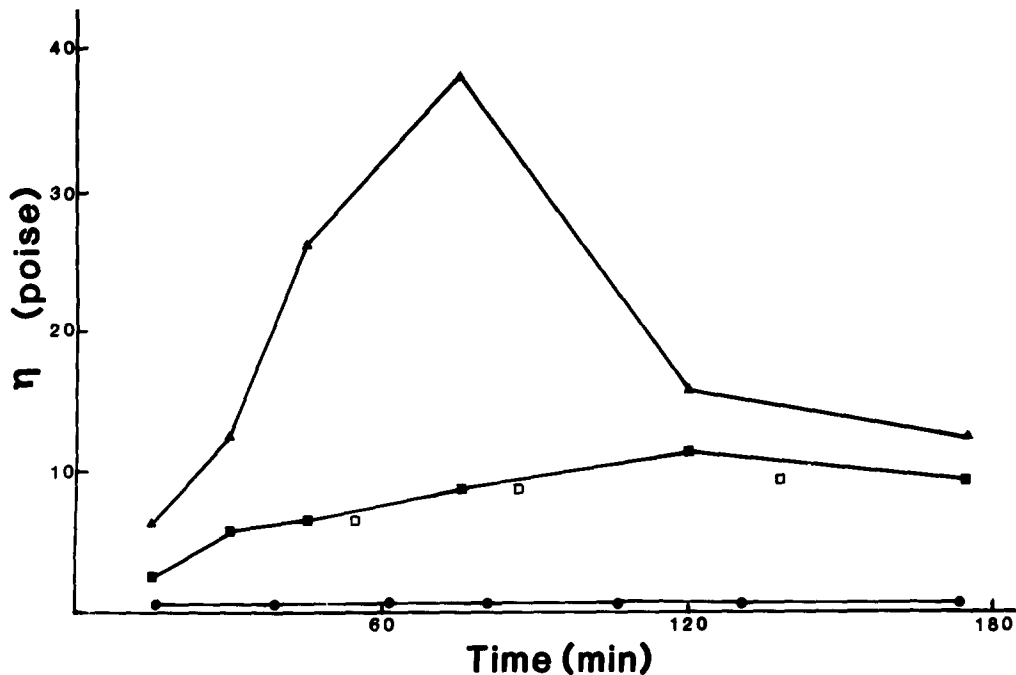
viscoelastometer. All of the preparations examined demonstrate a linear relationship between the logarithm of viscosity and the logarithm of frequency rate or equivalently, an exponential dependence of the viscosity on the frequency. This relationship as well as the absolute viscosity values are similar to data obtained previously by others, using a steady flow viscometer (20, 21). The effect of cytochalasin B is shown in Fig. 1 b. 5 μM of cytochalasin B decreased the dynamic viscosity of 2 mg/ml actin by 20% over the frequency range measured, but did not change the dependence of the viscosity on the frequency. As shown in Fig. 1 a, further purification of actin by Sephadex G-150 column chromatography increased the dynamic viscosity of 1 mg/ml actin from 20–60% over the frequency range measured. The viscosity varied from 400 to 3,200 centipoise (cp) over the frequency range measured as compared to 225 to 2,600 cp for less purified actin. In addition, the slope of the line for chromatographed actin was somewhat less than that of unchromatographed actin.

Fig. 2 shows apparent viscosity measurements of samples of the solutions described above obtained in the falling ball instrument. After the addition of salts to initiate polymerization, unchromatographed actin attained a constant apparent viscosity of ~60 cp after 20 min of incubation. In contrast, the apparent viscosity of chromatographed actin rose to a maximum value of 3,500 cp at 80 min and then fell to a final level of 900 cp. When sonicated fragments of polymerized chromatographed actin were added to chromatographed G-actin in a 1:20 weight ratio before initiation of assembly, the apparent viscosity increased monotonically with time after addition of KCl and MgCl₂, and reached a constant value at 2 h which was the same as that of chromatographed actin alone. The apparent viscosity of the sonicated chromatographed F-actin fragments, at a concentration of 1 mg/ml, after sonication was terminated, is also shown and had the same final apparent viscosity as monomeric chromatographed actin assembled in the presence of oligomers.

The viscosities measured by means of the falling ball viscometer of unchromatographed and chromatographed actin preparations in the presence of increasing amounts of actin-binding protein are shown in Fig. 3. Both actin and actin further
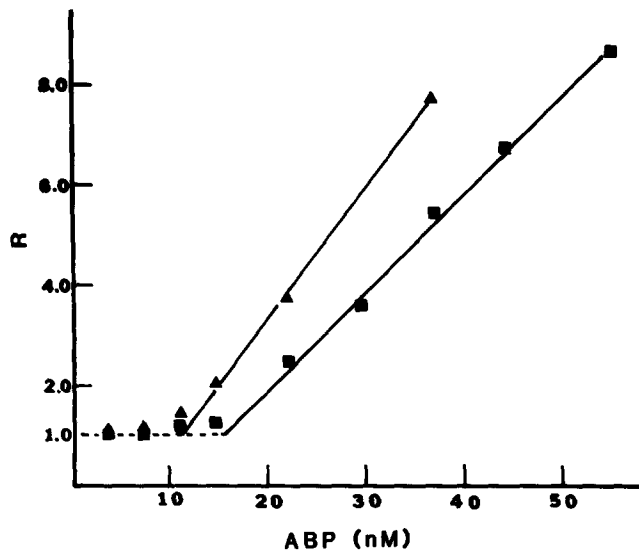

FIGURE 3 Gel points of 1 mg/ml actin solutions. The apparent viscosity of 1 mg/ml actin (▲) and 1 mg/ml chromatographed actin with sonicated F-actin (■) as described in Figure 1, in the presence of actin-binding protein (ABP) is plotted against ABP concentration. The apparent viscosities were measured by means of the falling ball viscometer and are plotted as R, the ratio of the viscosity of actin in the presence of ABP to the viscosity in its absence. Samples were diluted and polymerized as described in Fig. 1, for 2 h, ABP or Buffer was then added to the required final concentration, and the mixture was drawn into the capillary tube. Measurements were performed at 1 h.

purified by chromatography demonstrated a sharp increase in viscosity at a critical cross-linker concentration characteristic of an approach to gelation. Although the two curves are qualitatively quite similar, the absolute value of the data points from chromatographically purified actin lie much above those of actin. From the inflection points on the curves the weight average lengths, L_w, can be calculated and are 2.2 micrometers for unchromatographed actin and 3.1 micrometers for actin further purified by chromatography.

DISCUSSION

The findings presented in Fig. 1 demonstrate the frequency dependence of the dynamic viscosity of actin and are similar in form to results of shear rate-dependent measurements on actin obtained previously. The linear relationship between log ω and log $|\eta^*|$ as well as log γ and log η are universally observed properties of synthetic polymers in concentrated solutions at low to moderate rates of shear, referred to as power law behavior, and usually occurs over a limited range of rate of shear (22). This behavior can be satisfactorily predicted from topologic constraints on diffusion created by the entanglements between chains (23, 24). As the shear rate or frequency increases in these systems, the molecules disentangle, and the viscosity decreases. The theory predicts that the viscosity in the power law region is proportional to the polymer length raised to a power less than one, and experimental data concerning synthetic polymers supports the theory (11, 21). The findings presented here for actin also are in keeping with the principle that filament length is a determinate of actin's viscosity. The gel-point measurements of chromatographed and unchromatographed actin in the presence of macrophage actin-binding protein are indicative of differences in average filament lengths of ~40%. This difference can adequately explain the viscosity change in actin after gel-filtration chromatography. We have previously shown that 1 μM of cytochalasin B reduces the average filament length of 1 mg/ml of actin by magnitude similar to the change brought about by chromatography (18). The viscosity data shown in Fig. 1 concerning actin in the presence of 1 μM cytochalasin B is consistent with these earlier observations.

The falling sphere method is very useful for determining the viscosity of Newtonian fluids (25, 26). However, the analysis of its operation with fluids exhibiting power law behavior such as actin is considerably more complex (27, 28, 29). As noted in Figs. 1 and 2, the steady state apparent viscosity of actin with added nuclei measured by the falling ball viscometer is 15-fold higher after chromatography than before this step, which is quite different from the 40% difference obtained in the viscoelastometer. The discrepancy in results obtained by the two methods can readily be explained by the way in which the viscosity and shear rate interact with each other. When using the viscoelastometer, the frequency can be varied independent of the viscosity, whereas, the rate of strain in the falling ball viscometer varies with the inverse of the apparent viscosity, because their product must always be equal to the stress. The stress is proportional to the weight of the ball and is constant.

An estimate of the exaggeration of a difference in viscosity between two samples can be obtained by calculating the viscosity of actin which would correspond to a viscosity of chromatographed actin at a constant product of frequency and viscosity. Since there is a linear relationship between log $|\eta^*|$ and log ω for both actin preparations (Fig. 1) the data can be described by the standard linear expressions:

$$\text{Log } |\eta^*| = -0.79 \text{ Log } \omega + .14 \text{ Actin} \qquad \text{Eq. 3}$$

$$\text{Log } |\eta^*| = -0.69 \text{ Log } \omega + .41 \text{ Chromatographed actin} \quad \text{Eq. 4}$$

For a dynamic viscosity for chromatographed actin of 9 poise, which is about the same value obtained from the falling ball viscometer, we can calculate from Eq. 4 that there is a corresponding frequency of 0.16 sec^{-1}. If the product of the apparent viscosity and the frequency remain constant, we can substitute

the fact that:

$$\text{Log } |\eta^*| + \text{Log } \omega = \text{Log } (9 \times .16) = .165 \qquad \text{Eq. 5}$$

Into Eq. 3 and determine that the viscosity of actin would be 1.1 poise at a constant stress. However, the apparent viscosity of actin measured at a frequency of 0.16 sec^{-1} as obtained from Eq. 3 is 6.0 poise. Thus, neglect of the shear rate changes a 33% decrease in viscosity into a eightfold decrease. The apparent viscosity generated by this calculation is close to the value of 0.6 poise which was measured in the falling ball viscometer.

This comparison between a simple shear instrument and the more complex falling ball instrument neglects a further problem which is that the shear varies across the gap between ball and tube. Therefore, there will be a distribution of apparent viscosities across the gap, which will be quite difficult to predict. This problem has been elegantly analyzed by Sestak and Ambros (29) for the case of a ball which is almost the same size as its tube. Although this condition is only approximately fulfilled by the commonly used falling ball viscometer, the analysis can be used to estimate the magnitude of its contribution. These authors have found that the average shear rate for a given rate of ball movement is proportional to a function F(n) given by:

$$F(n) = 2 (1 + 2n)(1 + 2n)^2/n(2 + n)(2 + 3n) \qquad \text{Eq. 6}$$

where $n = 1 - d$, and d is the slope of the line obtained from a log-log plot of viscosity vs. frequency. The relationship predicts that an increase in the slope of the curve relating viscosity and shear will increase the average shear rate beyond what would be expected from the rate of ball movement. For example, the decrease of the slope from 0.79 to 0.67 corresponds to an additional 30% decrease in shear rate, which further widens the discrepancy calculated in the above analysis. This change will be further amplified by the fact that the ball is much smaller than the tube, so that the combined effects of these shear rate changes could generate an apparent viscosity very close to 0.6 poise.

In addition to documenting the amplification effect involved when the falling ball viscometer is used to analyze equilibrium viscosities of different actin preparations, we have also noted the amplication of a well-known kinetic phenomenon. The addition of oligomeric chromatographed actin to monomeric chromatographed actin before assembly eliminates a striking transient effect in the time course of viscosity changes measured during the polymerization of chromatographed actin in the falling ball viscometer. This latter effect can be explained in terms of the kinetics of actin polymerization (30–32). Gel filtration removes oligomeric actin and other nucleating factors, so that polymerization requires the spontaneous formation of actin nuclei, a step which occurs slowly under the experimental conditions. These nuclei rapidly elongate into a relatively small number of extremely long filaments which have a very high apparent viscosity. However, nuclei continue to form, and the filament length distribution slowly shifts towards the steady-state length distribution, which has a shorter average length and hence a lower viscosity. This transient is not evident during the assembly of unchromatographed actin or of actin containing sonic actin fragments, because both preparations have nuclei that hasten the attainment of the final length distribution. In many recent reports the time of viscosity measurements was either unspecified or was during the period in which these transients would have been taking place.

The preceding discussion can adequately explain the dis-

crepancy between the falling ball viscometer and the dynamic viscometer. However, there remains a similar discrepancy between apparent viscosities measured with the falling ball viscometer and the Ostwald viscometer. The effects of chromatography and cytochalasin B on the dynamic viscosity of F-actin were much smaller than on the apparent viscosity of F-actin in the falling ball viscometer, but were considerably greater than the barely detectable changes recorded with the Ostwald viscometer (5, 6, 18). An explanation of this discrepancy once again is an effect of rate of shear. The shear rate used in the common Ostwald viscometer is ~3 orders of magnitude higher than in the falling ball viscometer. Power law behavior is generally observable over a limited range of shear rate. Whereas the shear rate exerted by the falling ball viscometer used in recent work is within this range, that exerted in the Ostwald viscometers with which comparisons were made is probably outside this range. At high rates of shear, the molecules completely disentangle so that the viscosity is mainly a function of interactions between the polymers and the solvent and is somewhat insensitive to changes in molecular weight. At high shear rates the viscosity may become almost independent of shear rate, a phenomenon which has been designated as the "high-shear Newtonian limit" (33-36). The shear rate at which this phenomenon occurs decreases with increasing filament length, and may be in part responsible for the decreased power law slope for gel-filtered actin. Experimental data of this sort have been observed for relatively stiff synthetic chain molecules (37). In addition, and probably more important, the shear rates exerted by the Ostwald viscometer, degrade high molecular weight synthetic polymers and actin filaments (38, 39). This effect, called shear degradation, affects long filaments more than short ones and has the effect of making different length distributions more similar. The phonemena of the shear limit and especially of shear degradation explain qualitatively the decreasing proportionality between actin filament length and viscosity in the Ostwald viscometer as filament length increases (40) and render invalid previous attempts (including our own) (5, 18) to relate such viscosity values quantitatively to the molecular dimension of actin filaments.

The dynamic viscosity data obtained does indicate an increase in viscosity of actin after gel-filtration, but to a smaller extent than previously reported. The change is consistent with the increase in filament length derived from gel point data, and we do not believe that there is a need to postulate any other mechanism such as a specific filament-filament interaction to account for it. It should be emphasized that the evidence shows that highly purified actin is more viscous than less purified actin, but it is not valid to assume that this change reflects any increase in "gel-like" character. It may seem trivial to differentiate a very viscous actin sol from an actin gel, since both may appear quite "solid" on a macroscopic scale. However, the molecular interactions are not identical in these states and can be expected to have different influences in bulk flow, solvent, and solute exclusion properties and movements within a living cell. If F-actin molecules are by themselves bound together, it is unclear by what mechanism "gelation" factors can further change the rheologic properties of F-actin solutions. We feel that the unique rheologic properties of F-actin solutions are due to the extreme lengths of the filaments, which are far longer than most synthetic polymers and hence exhibit modes of behavior on a much longer time scale. In forthcoming papers we will further elucidate the properties of F-actin solutions and the mechanism of action of derived modulating factors.

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