Nucleotide Exchange, Structure, and Mechanical Properties of Filaments Assembled from ATP-actin and ADP-actin*

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Actin monomers with bound ATP, ADP, or fluorescent analogues of these nucleotides exchange the nucleotide on a second time scale, whereas filaments assembled from each of these species exchange their nucleotide with the solution at least 1,000 times slower than monomers. Filaments assembled from either ATP-actin or ADP-actin are indistinguishable by electron microscopy after negative staining. The dynamic elasticity and viscosity of filaments assembled from ATP-actin or ADP-actin or mixtures of these two species are the same over a wide range of frequencies. These observations do not support a recent suggestion (Janmey, P. A., Hvít?, S., Oster, G. F., Lamb, J., Stoss, T. P., and Hartwig, J. H. (1990) Nature 347, 95–99) that ATP hydrolysis within actin filaments stiffens the polymer and alters both their structure and affinity for nucleotides. The difference in observations between these two studies may be related to time-dependent changes in ADP-actin prepared in slightly different ways.

The adenine nucleotide bound in the central cleft of the actin molecule stabilizes the protein, but in spite of extensive research (for review see Korn et al., 1987; Pollard, 1990; and Carlier, 1991) the underlying function of the nucleotide and its hydrolysis in the assembly of actin filaments in live cells has remained an enigma for years (for review see Cooper, 1991). It is well established that when actin with bound ATP (referred to here as ATP-actin) polymerizes, the ATP on each molecule is hydrolyzed (Staub and Feuer, 1950) after its incorporation into a filament (Pollard and Weeds, 1984) in an irreversible reaction (Carlier et al., 1988) to ADP and inorganic phosphate (P?). The hydrolysis reaction may occur randomly on the polymerized subunits (Pollard and Weeds, 1984) or may be favored at the interface between a central region consisting of ADP-actin and newly added ATP-actin subunits near the ends of the filament (Carlier et al., 1987). The P? remains bound to the filament for some time (Carlier and Pantaloni, 1986), making rapidly growing filaments a heterogeneous mixture of subunits with ATP, ADP-P?, or ADP. Eventually the P? dissociates leaving filaments composed largely of ADP-actin with a few ATP- and ADP-P?-actin subunits near the ends.

The nucleotide composition of monomers has an easily measured impact on the elongation of actin filaments (for review see Korn et al., 1987). At the barbed end, we find by direct electron microscopic measurements that ATP-actin binds with a diffusion-limited rate constant of 107 M–1 s–1 and dissociates slowly, about 1 s–1 (Pollard, 1986; Drenckhahn and Pollard, 1986). (Similar values of these and other rate constants have been measured by other methods; for review see Korn et al., 1987.) In contrast, ADP-actin binds about 20% as fast and dissociates about 5 times faster than ATP-actin. ADP-P?-actin has a critical concentration similar to ATP-actin (Rickard and Sheterline, 1988; Wanger and Wagoner, 1987; Carlier and Pantaloni, 1988), but there are not yet any direct measurements of the elongation rate constants. The effect of nucleotide on growth at the pointed end is less clear. Both ATP- and ADP-actin associate at rates lower than expected for a diffusion-limited reaction (Pollard, 1986; Drenckhahn and Pollard, 1986). Both dissociate slowly, especially ADP-actin. The ratios of the association and dissociation rate constants give the critical actin monomer concentrations for elongation. In ATP with physiological concentrations of divalent cations, the critical concentration at the pointed end is substantially higher than at the barbed end. This should lead to a slow flux of subunits through the filaments from the barbed end to the pointed end driven by ATP hydrolysis.

These properties suggest that nucleotide hydrolysis might be used by cells to power subunit flux through actin filaments and possibly also to prepare the filaments for rapid disassembly at a time different from the time of assembly. In live cells actin subunits flux rapidly through filaments at the leading edge (Wang, 1985; Forscher and Smith, 1988), and many actin filaments turn over rapidly (Amato and Taylor, 1986; Theriot and Mitchison, 1991). Neither the rapid flux nor turnover appears to be compatible with the relatively slow dynamics of purified actin filaments in vitro, so we do not yet understand how the polymer dynamics in vivo are related to nucleotide hydrolysis (for review see Cooper, 1991). Excluded volume effects (Drenckhahn and Pollard, 1986) or accessory proteins could speed up subunit exchange in the cell.

Recent observations of Janmey et al. (1990), showing a possible effect of nucleotide hydrolysis on actin filament structure, mechanical properties, and nucleotide exchange, suggested a particularly exciting new hypothesis regarding the function of the nucleotide. Janmey et al. presented evidence that filaments assembled from ADP-actin differed from filaments assembled from ATP-actin in several ways even though both consist of ADP-actin subunits. Their ADP-actin filaments were irregular in contour and slightly larger in diameter than ATP-actin filaments. Nevertheless, solutions of the ADP-filaments had a much higher dynamic elastic modulus than ATP-actin filaments, a difference that the authors at-
tributed to the greater flexibility of the ADP-actin filaments, which caused them to be more entangled than the stiff rodlike ATP-actin filaments. The ADP-actin filaments exchanged their bound ADP faster than the ADP of filaments assembled from ATP-actin. The addition of ATP stiffened the ADP-actin filaments and gave them properties more like ATP-actin filaments. It was postulated that hydrolysis of ATP within an actin filament "traps ... the monomers ... conformationally and stores elastic energy" that might be "available for release by actin-binding proteins that transduce force or sever actin filaments."

Since this is the most interesting suggestion in years regarding the function of the bound nucleotide of actin, we thought that it is important to confirm the key observations of Janmey et al. (1990). In this paper we report new data on nucleotide exchange, filament structure, and mechanical properties of actin assembled from ADP-actin or ATP-actin.

**EXPERIMENTAL PROCEDURES**

**Protein Preparation**—Actin was purified from rabbit skeletal muscle and gel filtered in Buffer G on Sephadex G-150 (MacLean-Fletcher and Pollard, 1980). Buffer G consists of 2 mM Tris-CI, pH 8.0, at 25°C, 0.2 mM ATP, 0.5 mM dithiothreitol, 0.1 mM CaCl₂, 1 mM sodium azide. Mg-ADP-actin was made by the method of Pollard (1986) using soluble hexokinase, or, for the nucleotide exchange experiments, by addition of Mg-ADP using hexokinase-agarose beads as follows. One milliliter of actin at a concentration of 50-50 μM was mixed gently with 50 μl of Dowex 1 (Bio-Rad AG-X2 washed in 2 mM Tris, pH 8.0) for 5 min at 4°C. The Dowex binds free nucleotides. The Dowex was removed by centrifugation at 14,000 × g for 30 s, and 852 μl of nucleotide-free actin monomers from the supernatant was added to a microcentrifuge containing 100 μl of packed hexokinase-agarose beads. The sample was mixed gently for 5 min at 0°C, and the hexokinase-agarose beads, the Mg-ADP-actin in the supernatant was removed by centrifugation at 14,000 × g for 30 s, and 852 μl of nucleotide-free actin monomers from the supernatant was added to a microcentrifuge containing 100 μl of packed hexokinase-agarose beads (Sigma H-2005; washed with 2 mM Tris-CI, pH 8.0, 0.5 mM dithiothreitol; final concentration 2 units/ml), 5 or 10 μl of 100 mM ADP (final concentration 0.5 or 1 mM), 8 μl of 10 mM MgCl₂ (final concentration 80 μM), 20 μl of 10 mM EGTA1 (final concentration 200 μM), and 10 μl of 100 mM glucose (final concentration 1 mM). The sample was mixed gently for 4 h at 0°C. After pelleting the hexokinase-agarose beads, the Mg-ADP-actin in the supernatant was stored on ice and used the same day. Etheno-ADP (Molecular Probes, Inc.) was exchanged onto the actin in the same way using a final concentration of etheno-ADP of 0.5 mM. Etheno-ADP-actin is not stable for more than a few hours at 0°C based on a progressive loss in the amplitude of the signal when ATP is exchanged for the etheno-ADP. Etheno-ATP was exchanged onto the actin in the same way with the omission of the hexokinase beads using a final concentration of 0.5 mM etheno-ATP.

**Actin Nucleotide Exchange Experiments**—The binding or dissociation of etheno-ATP or etheno-ADP, A is actin, eN is the high fluorescence form of the nucleotide analog bound to actin, and N is ATP or ADP. The simulations were initiated by adding the second nucleotide to an equilibrium mixture of actin and the first nucleotide. The association rate constants were assumed to be 10 μm⁻¹ s⁻¹ (Nowak et al., 1988). We searched by trial and error for the single set of dissociation rate constants, one for each of the four nucleotides, that allowed the kinetic simulations to fit simultaneously the full-time course of the fluorescence changes in all four experiments and all actin concentrations in Fig. 1. Initially the ratios of these dissociation rate constants were constrained by the ratios of the corresponding Kd values as determined above but had to be adjusted slightly during the simulations to achieve a good fit to all of the data. The optimal rate constants are given in the legend of Fig. 1, and simulations 1 are illustrated in Fig. 1a as continuous curves.

**Actin Polymerization**—Some of the actin was modified with pyrenyl-iodoacetamide (Molecular Probes, Inc.) and used to measure actin polymerization (Pollard, 1984). The critical concentration for polymerization was determined from the dependence of the steady-state fluorescence on actin concentration (Pollard, 1984). Having established that actin filaments do not exchange bound nucleotide at an appreciable rate, we also used the extent of fluorescent nucleotide exchange as a function of the concentration of actin as a new method to measure the critical concentration of monomers in the presence of filaments.

**Electron Microscopy**—Actin filaments on glow-discharged carbon films were negatively stained with 1% uranyl acetate (Cooper and Pollard, 1983). Four grids each of ATP-actin and ADP-actin filaments were coded and randomized before one author took micrographs at two blinded observers.

**Rheological Measurements**—The dynamic viscosity and elasticity were measured in a cone and plate rheometer with small amplitude oscillations as described by Sato et al. (1985). ADP-actin was made by the method of Pollard (1986). Samples were degassed to avoid bubbles during the long incubations. Actin stocks were warmed to 25°C and diluted with ADP or ATP buffer and concentrated salts to give final concentrations of 34 μM actin, 50 mM KCl, 1 mM MgCl₂, 0.5 mM EGTA, 0.2 mM nucleotide, 10 mM imidazole, pH 7.0. Samples were immediately transferred to the rheometer and incubated at 25°C for 8-12 h to attain an equilibrium configuration before measurements were made of the dynamic viscosity and elasticity.

1The abbreviation used is: EGTA, [ethylenebis(oxyethyleneenitrilo)tetraacetic acid.}
RESULTS

Preparation of Actin-Nucleotide Complexes—Actin with bound Mg\(^{2+}\) and ADP or etheno-ADP prepared by exchange in a low ionic strength buffer containing Mg\(^{2+}\), EGTA, glucose, and hexokinase had a critical concentration 10–20 times higher than the corresponding ATP-actins (Table I). The hexokinase acts as a scavenger for any ATP dissociated from the actin or synthesized from ADP by contaminating adenylate kinase (Pollard, 1986; Gershman et al., 1989). Soluble hexokinase was left in the ADP-actin preparations used for rheological studies to remove any ATP generated during the equilibration and measurements.

To measure the exchange of ADP bound to the actin for ATP from the buffer, it was necessary to remove the hexokinase from the actin. This was accomplished by a new method using hexokinase immobilized on agarose beads. The hexokinase activity that could be achieved in the exchange reaction using the commercial preparation of hexokinase-agarose was about 10 times lower than the concentration of soluble hexokinase known to be effective in producing ATP-free actin in previous studies (Pollard, 1986). To reduce the need for high hexokinase activity, free ATP was removed from the starting sample of actin before adding ADP and the immobilized enzyme. In steady-state critical concentration experiments the resulting Mg-ADP-actin has the same critical concentration that made with soluble hexokinase (Table I). Thus the two preparations of Mg-ADP-actin have the same ratio of dissociation and association rate constants for polymer elongation. It is formally possible that the absolute values of these rate constants may differ for the two preparations. Since the critical concentration is very sensitive to low concentrations of ATP-actin in ADP-actin (Ohm and Wegner, 1991), these results are strong evidence that the hexokinase-agarose efficiently removes all of the ATP from the ADP-actin preparation like soluble hexokinase (Pollard, 1984).

Nucleotide Exchange on Actin Monomers—We first carried out a comprehensive set of nucleotide exchange experiments with actin monomers (Fig. 1) to establish a quantitative mechanism that would allow us to interpret experiments with more complex mixtures containing both monomers and filaments. Others have investigated nucleotide exchange by actin monomers (see for example Kuehl and Gergely, 1969; Waechter and Engel, 1975; Frieden and Patane, 1988; Nowak et al., 1988), but not for all of the relevant nucleotides and not in a buffer that approximates physiological ionic conditions.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Critical concentrations for actin polymerization</th>
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<td>Conditions: 50 mM KCl, 1 mM MgCl(_2), 1 mM EGTA, 0.2 mM nucleotide, 0.5 mM dithiothreitol, 10 mM imidazole (pH 7.0). The underlined values were obtained with samples of actin prepared with hexokinase-agarose beads. The other ADP-actin samples were prepared with soluble hexokinase. The samples used for elongation rate and steady-state polymer concentration measurements contained 5% pyrene-labeled actin.</td>
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<td>Method</td>
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<tr>
<td>Elongation rate</td>
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<td>Steady-state polymer concentration</td>
<td>0.10</td>
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<tr>
<td>Nucleotide exchange</td>
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Although the exchange of actin-bound nucleotide is relatively slow in calcium-containing solutions (Gershman et al., 1989), ATP, ADP, etheno-ATP, and etheno-ADP all exchange on a second time scale in buffers with Mg\(^{2+}\) and EGTA (Fig. 1). Under these conditions, Mg\(^{2+}\) is bound to the high affinity divalent cation site (Gershman et al., 1986) in the cleft of the actin molecule (Kabsch et al., 1990). The time course is essentially the same when the Mg-EGTA buffer has either a low ionic strength favoring depolymerization of the actin or high enough concentrations of KCl and Mg\(^{2+}\) to strongly favor polymerization of the actin molecules.

Simulation of the time course of these exchange reactions allowed us to select dissociation rate constants for each of the four nucleotides (Fig. 1 legend) that together account the kinetic data from four different types of nucleotide exchange experiments (Fig. 1). We used the two-step mechanism explained under "Experimental Procedures." The simulation approach allowed us to model the reactions without simplifying assumptions about irreversibility used in earlier analytical solutions (Waechter and Engel, 1975). Although the nucleotide exchange rate constants are very sensitive to experimental conditions (Frieden and Patane, 1988), our complete set of values for the rate constants is in general agreement with literature values obtained for subsets of these constants under somewhat different conditions (Kuehl and Gergely, 1969; Waechter and Engel, 1975; Nowak et al., 1988).

Nucleotide Exchange by Polymerized Actin—Samples of polymerized actin assembled from monomers with bound ATP, ADP, etheno-ATP, or etheno-ADP exchanged a small fraction of their nucleotide on a second time scale, but the rate of exchange by most of the actin in these samples was too slow to detect in 20 min (Fig. 2). This confirms for all nucleotides previous observations on filaments assembled from ATP-actin (Martonosi et al., 1960).

The time course for the exchangeable fraction of nucleotide in each of these polymerized actin samples was similar to samples of monomer without filaments, and the amplitude of the signal was equal to or slightly less than that predicted by the critical concentration of monomers measured independently by steady-state or kinetic methods. Beyond this signal arising from exchange of nucleotide by the monomers, there was no additional reproducible signal attributable to nucleotide exchange by the polymeric actin over the time course of our experiments. In the experiments with ATP-actin plus etheno-ATP, we detected no exchange of nucleotide by polymer concentrations up to 50 times the critical concentration of monomers, where exchange, even at a low rate, would have been obvious (Fig. 2A). The signal to noise ratio is even better with etheno-ATP-actin reacted with ATP. In these experiments, polymer concentrations 60 times higher than the critical concentration did not exchange over a period of 20 min (Fig. 2B). In the experiments with ADP-actin filaments the critical concentration is much higher, so the highest polymer concentration tested was six times the monomer concentration. Nevertheless, no nucleotide exchange of the polymerized actin was detected over times greater than 10 half-times for exchange of nucleotide on the monomers in the same sample (Fig. 2C). Similar results were obtained with filaments of etheno-ADP-actin where the signal to noise ratio was even better (Fig. 2D).

Given the sensitivity of these assays, the half-time for the exchange of nucleotide on the subunits of actin filaments is more than 1,000 times greater than the half-times for the exchange of the same nucleotide bound to actin monomers. This conclusion holds true for filaments assembled from ATP-actin, ADP-actin, etheno-ATP-actin, or etheno-ADP actin.
ATP- or ADP-Monomers—We detected no reproducible differences in the viscous or elastic moduli of actin filaments assembled from ATP-actin or ADP-actin over a wide range of frequencies (Fig. 4). Filaments assembled from mixtures of ATP- and ADP-actin (made by converting most but not all of the buffer ATP to ADP) had similar mechanical properties.

Electron Microscopy of Actin Filaments Assembled from ATP- or ADP-Monomers—Although there is always some heterogeneity in the appearance of actin filaments prepared for electron microscopy by negative staining, we could not detect any systematic differences in the gross appearance of actin filaments assembled from ATP-actin (Fig. 3, A–C) or ADP-actin (Fig. 3, D–F). In particular, all of the grids contained filaments with smooth, gently curving contours. Most of the grids also contained some irregular filaments, but blinded observers could not distinguish two populations of grids made with ATP-actin or ADP-actin. The quality of the stain varied from one grid square to the next but was not related to the nucleotide. One of four grids with ADP-actin had considerable lateral aggregation of the filaments, but this was not characteristic of the other ADP grids. These observations rule out gross differences in filaments of ATP-actin and ADP-actin but do not preclude subtle differences in the packing or shape of the subunits that might be detected by high resolution reconstructions.

Mechanical Properties of Actin Filaments Assembled from ATP- or ADP-Monomers—We detected no reproducible differences in the viscous or elastic moduli of actin filaments assembled from ATP-actin or ADP-actin over a wide range of frequencies (Fig. 4). Filaments assembled from mixtures of ATP- and ADP-actin (made by converting most but not all of the buffer ATP to ADP) had similar mechanical properties.

DISCUSSION

In a physiological buffer containing Mg\(^{2+}\), KCl, and EGTA, a single set of rate constants can account for the time courses of a wide range of nucleotide exchange experiments (Fig. 1), so we are confident that these values are reliable. All are based on a reasonable assumption that the association rate constants are 10 \(\mu M^{-1} s^{-1}\), a value cited by Nowak et al. (1988) from unpublished work.

Four adenine nucleotides exchanged on a second time scale from actin monomers, but we detected very little exchange on actin filaments over much longer periods of time even in experiments with high concentrations of filaments (Fig. 2). Conservatively, we estimate that the nucleotide bound to actin filaments polymerized from either ATP- or ADP-actin exchanges 1,000 times slower than ATP or ADP on actin monomers. This exchange rate for subunits within filaments is an upper limit, since some nucleotide exchanges into polymers indirectly because of nucleotide exchange on monomers that then exchange on and off the ends of filaments. The slow rate of nucleotide exchange by polymerized actin is consistent with the atomic model of the actin filament (Holmes et al., 1990) where subunit contacts along the long pitch helix appear to block the exit of the nucleotide from its binding site as well as constraining the type of interdomain motions that open
Phase was about with ours. They capped the barbed end of the filaments. They exchanged bound nucleotide more rapidly than ATP-ADP-actin filaments (Pollard, 1986), is not necessary to postulate rapid exchange of nucleotide by the flux of subunits through filaments. Our calculation of their subunit exchange rate (0.1 nM subunits/2 mM KCl with MgCl₂ the maximum rate was about 0.1 nM s⁻¹) was consistent with the rate of subunit flux (about 0.1 s⁻¹) calculated from the elongation rate constants (Pollard, 1986), so it is not necessary to postulate rapid exchange of nucleotide by subunits within the polymers. These conclusions are consistent with ours.

Janmey et al. (1990) reported that solutions of ADP-actin filaments exchange bound nucleotide more rapidly than ATP-actin filaments. They capped the barbed end of the filaments to eliminate the slow flux of subunits observed by Wang and Taylor (1981). From the the partial time courses of nucleotide exchange in polymeric actin reported by Janmey et al. we estimate first-order rate constants of 0.005 s⁻¹ for ADP-actin and 0.001 s⁻¹ for ATP-actin. Their signal came from the binding of etheno-ATP, but these values should approximate the dissociation rate constants for ADP and ATP. Both values are about 5-fold lower than our dissociation rate constants for ADP and ATP from monomeric actin, a difference that is expected because of the Ca²⁺ in their buffer which slows the rate of nucleotide dissociation (Frieden and Patane, 1988). We suggest that their signal arose in large part from the critical concentration of monomers in their filament samples, as in our experiments in Fig. 2. The reason is that Janmey et al. calibrated their fluorescence signal by assuming that 100% of the nucleotide in samples of filaments exchanged in 20 h. They calculated the fluorescence increment per molecule of actin-bound nucleotide from the total actin concentration and the fluorescence change at that time. Twenty hours is probably inadequate for complete equilibration of nucleotide with actin, a difference that is expected because of the Ca²⁺ in their buffer which slows the rate of nucleotide dissociation (Frieden and Patane, 1988). We suggest that their signal arose in large part from the critical concentration of monomers in their filament samples, as in our experiments in Fig. 2. The reason is that Janmey et al. calibrated their fluorescence signal by assuming that 100% of the nucleotide in samples of filaments exchanged in 20 h. They calculated the fluorescence increment per molecule of actin-bound nucleotide from the total actin concentration and the fluorescence change at that time. Twenty hours is probably inadequate for complete equilibration of nucleotide with actin, a difference that is expected because of the Ca²⁺ in their buffer which slows the rate of nucleotide dissociation (Frieden and Patane, 1988). We suggest that their signal arose in large part from the critical concentration of monomers in their filament samples, as in our experiments in Fig. 2. The reason is that Janmey et al. calibrated their fluorescence signal by assuming that 100% of the nucleotide in samples of filaments exchanged in 20 h. They calculated the fluorescence increment per molecule of actin-bound nucleotide from the total actin concentration and the fluorescence change at that time. Twenty hours is probably inadequate for complete equilibration of nucleotide with actin, a difference that is expected because of the Ca²⁺ in their buffer which slows the rate of nucleotide dissociation (Frieden and Patane, 1988).
Actin Nucleotides


FIG. 4. Comparison of the rheological properties of ATP- and ADP-actin filaments. Actin filaments at were analyzed by oscillation in a cone-plate rheometer at 25 °C over a wide range of frequencies. Conditions: 34 μM ATP-actin (circles); 34 μM ADP-actin (squares). The filled symbols represent the elastic modulus (G') in dynes/cm², and the open symbols represent the dynamic viscosity in poise.

the critical concentrations are determined by the pointed ends where the ATP-actin and ADP-actin differ only by a factor of 2–3 (Coue and Korn, 1985; Pollard, 1986).

We used nucleotide exchange by monomers and competitive nucleotide binding experiments (see “Experimental Procedures”) to calibrate the absolute fluorescence increment for each nucleotide exchange pair. In our experiments with mixtures of filaments and the critical concentration of monomer, only the critical concentration of actin monomers exchanged rapidly. Even without capping the barbed ends, no nucleotide exchange pair gave a substantial fluorescence signal from filaments during the 20-min duration of our experiments. Because the nucleotide exchange rate by polymerized actin is so slow, a new experimental design will be required to detect any subtle differences in nucleotide exchange rates of filaments assembled from ADP- and ATP-actin.

We found no difference in the electron microscopic appearance (Fig. 3) or mechanical properties (Fig. 4) of actin filaments assembled from ADP- or ATP-actin. Our observations do not exclude small differences in the structure of ADP- and ATP-actin filaments that may exist at the atomic level, but both the microscopy and rheology differ from the report of Janmey et al. (1990). First, our ADP-actin filaments have smooth, not irregular, profiles just like ATP-actin filaments.
Second, our ADP-actin filaments and ATP-actin filaments had similar mechanical properties, whereas the ADP-actin filaments of Janmey et al. were about 10 times more rigid than their ATP-actin filaments. Furthermore, the absolute values of the elastic moduli of filaments prepared from fresh actin are much lower in our laboratory than Janmey’s, an unresolved, long-standing difference (see Sato et al., 1985; Janmey et al., 1988). The experimental design differed in two ways: (a) methods to prepare the actin, and (b) the inclusion of gelsolin in the rheological experiments of Janmey et al. to control the length of the filaments. Differences in the actin rather than the gelsolin seem more likely to account for the difference in rigidity.

The differences in the observations between the two laboratories have not yet been resolved but may lie in the preparation of the ADP-actin. Our current methods take advantage of rapid exchange of nucleotide by Mg-actin (Gershman et al., 1989) to make ADP-actin quickly in the cold in an effort to avoid an inevitable, time-dependent (k = 2 × 10−5 s−1 at 0 °C) conformational change that can be detected in ADP-actin by sulphydryl titration of Cys-10 (Drewes and Faulstich, 1991). We never freeze purified actin preparations. Janmey et al. used a slower, early method from our laboratory (Pollard, 1984) to prepare ADP-actin from frozen stocks. Ca-ATP-actin is incubated with hexokinase and glucose at room temperature for several hours. These ADP-actin preparations are not completely stable (Pollard, 1984), perhaps because of the conformational change. It was necessary to incubate their ADP-actin for 2 h at room temperature before the differences with ATP-actin became apparent. The Cys-10 conformational change should have occurred on part of the ADP-actin prepared by this method and may account for the substantial differences in the properties of the ADP-actin in the two laboratories. It is particularly interesting that the structural differences in ADP-actin filaments observed by Janmey et al. (1990) were reversed rapidly by ATP just like the Cys-10 conformational change (Drewes and Faulstich, 1991). O’Donoghue et al. (1992) also observed that ADP-actin prepared by our 1984 method has a higher viscosity than ATP-actin.

Given that ADP- and ATP-actin filaments are the same by our criteria, new evidence will be necessary to confirm the hypothesis that nucleotide hydrolysis within actin filaments is used to modulate their mechanical properties. Rather, we expect that nucleotide hydrolysis is used to drive subunit flux through the filaments and to prepare filaments for rapid disassembly at a time subsequent to their assembly.

Acknowledgments—We thank Dr. Pascal Goldschmidt-Clermont for suggesting the use of the hexokinase-agarose beads for making ADP-actin and Dr. Paul Janmey for exchanging ideas, data, and proteins in our ongoing effort to reconcile our experimental results.

REFERENCES