Nucleotide exchange and rheometric studies with F-actin prepared from ATP- or ADP-monomeric actin

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ABSTRACT It has recently been reported that polymer actin made from monomer containing ATP (ATP-actin) differed in EM appearance and rheological characteristics from polymer made from ADP-containing monomers (ADP-actin). Further, it was postulated that the ATP-actin polymer was more rigid due to storage of the energy released by ATP hydrolysis during polymerization (Janmey et al. 1990. *Nature* 347:95–99). Electron micrographs of our preparations of ADP-actin and ATP-actin polymers show no major differences in appearance of the filaments. Moreover, the dynamic viscosity parameters G′ and G″ measured for ATP-actin and ADP-actin polymers are very different from those reported by Janmey et al., in absolute value, in relative differences, and in frequency dependence. We suggest that the relatively small differences observed between ATP-actin and ADP-actin polymer rheological parameters could be due to small differences either in flexibility or, more probably, in filament lengths. We have measured nucleotide exchange on ATP-actin and ADP-actin polymers by incorporation of α-^{32}P-ATP and found it to be very slow, in agreement with earlier literature reports, and in contradiction to the faster exchange rates reported by Janmey et al. This exchange rate is much too slow to cause "reversal" of ADP-actin polymer to ATP-actin polymer as reported by Janmey et al. Thus our results do not support the notion that the energy of actin-bound ATP hydrolysis is trapped in and significantly modifies the actin polymer structure.

INTRODUCTION

It is now recognized that actin filaments have a central role in a wide variety of cellular processes, including cell shape determination, cell motility, and cytoplasmic consistency. These cellular processes, which are usually regulated by actin-associated proteins, often involve actin filament length changes or changes in the shape of actin filaments, i.e., flexible or rigid in appearance (1, 2). Recently, Janmey et al. (3) have reported that the shape and length of an actin filament can change with conditions. They suggested that F-actin formed from monomeric G-actin containing tightly-bound ATP is more rigid than actin polymers formed from monomer units containing ADP as bound nucleotide.

In principle, changes in the shape of an elongated filament, as F-actin is thought to exist in solution, would be expected to be detected by changes in mechanical properties of the polymer. Earlier studies of the rheological characteristics of actin have established that monomeric actin behaves like a Newtonian fluid having a viscosity very similar to that of its aqueous solvent (4–6). On the other hand, F-actin exhibits viscoelastic behavior that is elastic due to immobilization of the elongated actin filaments because of extensive overlap of rotational domains, although the filaments do not otherwise interact (7). Measurements of the dynamic storage modulus, G′, and the dynamic loss modulus, G″, over a range of applied frequencies, allow separation of energy storage and dissipative processes in a solution of F-actin and thus permit a description of the mechanical properties of the polymer solution (7, 8).

The report by Janmey et al. (3) suggested that polymer prepared from ATP-actin monomers is a rigid polymer, while polymer prepared from ADP-actin monomers is highly flexible. Since both types of polymer contain primarily ADP as the polymer-bound nucleotide, a model was proposed suggesting that for F-actin prepared from ATP-actin monomer, the energy of the ATP hydrolyzed during polymerization is stored in the protein structure by locking the polymer into a rigid conformation. The data supporting this notion included electron micrographs of F-actin prepared from both ATP and ADP monomers showing differences in filament rigidity, rheological experiments showing large differences between the two polymers, nucleotide exchange data which suggested that ATP can rapidly exchange into F-actin prepared from ADP-actin monomer, and raw data from dynamic light scattering experiments showing differences between the two polymers which were attributed to flexibility differences.

We have investigated this interesting idea using experimental approaches similar to those used by Janmey et al. (3). However, we can find no compelling evidence supporting a significant difference in flexibility between actin filaments polymerized from ATP- or ADP-monomer

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(9). The original suggestion that F-actin can store the energy of hydrolysis (3) relied heavily on evidence that ATP could exchange into polymer actin with a half time on the order of 1000 s. This rapid exchange was in sharp contrast to previous measurements of nucleotide exchange into polymer (10, 11). We also find no evidence for such a rapid exchange. A recent study by Pollard et al. (3) reaches a similar conclusion.

MATERIALS AND METHODS

Materials

All reagents were analytical grade. ATP, ADP, EDTA, and hexokinase were purchased from Sigma Chemical Co. and α-^{32}P-ATP from Amersham.

Protein preparation

Actin was prepared in two locations: Boston University School of Medicine (BUSM), Boston, MA, for the rheological measurements done in Boston, and Department of Veterans Affairs Medical Center, Albany, NY, for all other measurements. The methods used for actin purification by the two laboratories were not the same but the resulting preparations displayed similar rheological properties.

Actin was extracted from rabbit skeletal muscle acetone powder as prepared by the method of Szent-Gyorgyi (13). At BUSM the purification of actin was accomplished by the method of Spudich and Watt (14) and, following procedures similar to Janmey et al. (3), the actin was resuspended in 0.5 mM ATP (ATP-actin) or 0.5 mM ADP (ADP-actin), 0.2 mM $CaCl_2$, 0.2 mM DTT, and 5 mM Tris, pH 7.4. The resuspended actin was dialyzed against its respective ATP or ADP containing buffer for 40 hours with three changes of 2 liters each. The samples were then divided into 0.5 ml aliquots and quick frozen in liquid nitrogen. Previously published procedures (15) were used in the Albany laboratory except that the eluent for the final chromatography step contained 0.2 mM ATP, 0.02 mM $CaCl_2$, 0.01% NaN_3, and 5 mM HEPES, pH 7.0. ATP-actin and ADP-actin were first prepared exactly by the method of Janmey et al. (3). The contaminant ATP level in the ADP-actin dialysate was similar to that of Janmey et al., but further analysis revealed that about 65% of the ADP-actin monomer prepared using these procedures contained ATP. ADP-actin was also prepared using the hexokinase/glucose method (16) because of the high affinity of actin for ATP (K_{dADP}/K_{dATP} = 200) and the presence of contaminant ATP in most commercial ADP preparations. This method treated Mg-ATP-actin at pH 8.0 on ice with hexokinase and glucose for one hour, and was then brought to pH 7 before use. These samples were not frozen and ATP-actin samples were used within 4 days, while ADP-actin samples were used on the day of preparation.

The presence of ATP in ADP-actin preparations was assayed using a luciferin/luciferase assay mixture obtained from Sigma Chemical Co. (Product number L 0633). Briefly, 20 μL of assay reagent, which had been diluted to 20 mg/ml with water, was added to 0.5 ml of sample supplemented with 1 mM $MgCl_2$ in a 2 mm $\times$ 10 mm quartz cuvette. The emitted light was immediately measured for 50 s. at 540 nm with a 20 nm bandpass filter. The integrated light intensity was compared to standard ATP solutions in the 1–10 μM concentration range. The assay was accomplished before and after heat denaturation to determine both free and bound ATP concentrations as well as to destroy the hexokinase activity in samples containing hexokinase and glucose.

Gelsolin was purified from human serum by the method of Cooper et al. (17) at BUSM.

Nucleotide exchange experiments

Exchange of ATP into F-actin prepared from ADP-actin monomer and ATP-actin monomer was monitored by α-^{32}P-ADP release. Monomeric Ca-actin, 20 μM in 5 mM HEPES, pH 7.0, 0.02 mM $CaCl_2$, 20 μM ATP was first labeled with α-^{32}P-ATP by overnight incubation at 0°C. The actin was converted to Mg-actin by the addition of $MgCl_2$ to 50 μM and EDTA to 100 μM. After further incubation for 1 h., the preparation was divided into two portions; one half was polymerized with 0.1 M KCl and 2 mM $MgCl_2$ (final concentration) (F-ATP-actin) and the other half was converted to ADP-actin using the hexokinase and glucose method reported previously (16), before being polymerized with 0.1 M KCl and 2 mM $MgCl_2$ (F-ADP-actin). Excess nucleotide was then removed by the addition of Dowex AG1X8 ion exchange resin, followed by filtration to remove the Dowex, or by centrifugation at 100,000 g for 2 h, followed by pellet resuspension. Both methods of removing excess free nucleotide from the actin preparations resulted in about 1 μM free nucleotide in the polymer preparations.

At t = 0, 0.5 mM unlabeled ATP was added to ATP-actin polymer and ADP-actin polymer and aliquots were removed over the next 14 days. Each aliquot was centrifuged in an Airfuge at 100,000 g for 10 min. and the amount of radioactivity in the supernatant and pellet determined. The loss of α^{32}P-ADP from the pellet was matched by the simultaneous increase of α^{32}-ADP in the supernatant for each aliquot removed in the time course. Both protein and ATP concentrations in the supernatant fractions were constant in value over the 14-day time course.

Rheology

Rheological properties of actin samples were measured under a variety of conditions at each of two facilities. At BUSM, the dynamic storage and loss modulus in simple shear, $G'(\omega)$ and $G''(\omega)$, respectively, were measured using a mica-plate viscoelastometer as previously described (6, 7, 18, 19). The dynamic moduli were measured by either applying a sinusoidally varying stress and observing the resultant strain or applying a stepped stress and calculating the Fourier transform of the resultant time dependent strain (8).

At Union College, the dynamic moduli were determined using a Rheometrics RFS II Fluids Spectrometer. This instrument is very similar to that used by Janmey et al. (3) except that the Union College instrument uses a titanium 5.0 cm diameter standard cone and plate arrangement rather than parallel plates of stainless steel.

In both laboratories, ADP- and ATP-actin samples were diluted using the appropriate nucleotide-containing buffer, to final concentrations of 38 and 34 μM, respectively, accounting for the differences in critical concentrations. Appropriate amounts of gelsolin were added and polymerization of the actin/gelsolin mixture was induced by adjusting the salt concentration to 0.15 M KCl and 2 mM $MgCl_2$, chosen in accord with Janmey et al. (3). In most cases the sample was immediately placed in the viscoelastometer, although no differences were observed in steady-state results using the RFS II if the samples were placed on the bottom plate after polymerization for one hour.

The BUSM samples were then left undisturbed in the viscoelastometer for 60 minutes, at which time the dynamic moduli were obtained at a frequency of 0.03 Hz. An additional measurement was made at 90 minutes and if the second measurement was within 5% of the first determination, the frequency spectra measurements were initiated. An additional dynamic measurement at 0.03 Hz was performed after the frequency spectrum was obtained and was found to differ by less than 5% from the initial 90 minute result.

The Union College samples were typically monitored by repeated dynamical measurements at 1 or 2% strain and 1 rad/s until the dynamic storage and loss moduli reached a plateau, which occurred within 1–2 hours for samples with gelsolin present. Frequency spectra were then obtained and a final measurement at 1 rad/s reproduced the initial plateau value within 5%.

Electron microscopy

Electron microscopy was performed at SUNY Albany using a Zeiss 902 transmission EM (Carl Zeiss, Thornwood, NY). Both ATP- and

ADP-actin polymer samples were applied at a concentration of 2.4 to 10 μM to glow-discharged carbon stabilized formvar coated grids and were negatively stained with 1% uranyl acetate. The samples were polymerized at 24 μM actin concentration with 2 mM $MgCl_2$ and 0.1 M KCl and diluted into the same buffer just prior to application to the grids. Actin filaments in electron micrographs were scored for flexibility by two blinded observers.

RESULTS

Electron microscopy

Fig. 1 shows electron micrographs of F-actin prepared from ATP-actin monomer (upper) and from ADP-actin monomer (lower). We note, however, that it proved more difficult to prepare good quality sample grids for the filaments prepared from ADP-actin monomer, perhaps due to the relatively large amount of monomer protein in solution as a result of the larger critical concentration. This is manifest in the obvious background difference between the two micrographs. Variations in preparing the grids for microscopy showed that the "bubble" pattern seen in the lower field is due to a higher monomer actin concentration and that the filaments are unaffected by the concentration of monomer when the grids were formed. No major differences in the filament ultrastructure, and in particular, no differences in filament flexibility, were observed.

Rheology

The frequency dependence of both the dynamic storage, G′, and dynamic loss, G″, moduli measured at BUSM on F-ATP-actin and F-ADP-actin solutions, using samples prepared following the Janmey et al. prescription, with gelsolin added at a ratio of 1:1,000, is shown in Fig. 2. The frequency spectra were measured using the mica plate viscoelastomer. Note that the shapes of the G′ and G″ curves for the two different preparations are very similar and the values of ADP-actin parameters are within about twice those of ATP-actin over the entire frequency range. These relative differences between ADP- and ATP-actin are five- to ten-fold lower than reported by Janmey et al. (3), and moreover, the absolute values of the individual moduli are between 20- and 100-fold lower than reported by Janmey et al. (3). Also, note the absence of the strong frequency dependence of G″ observed by Janmey et al. for their F-ADP-actin.

Frequency spectra of ATP- and ADP-actin polymer were also measured at Union College using the Rheometrics Fluids Spectrometer RFS II as shown in Fig. 3. The actin samples were first prepared using exactly the same procedures as described in Janmey et al. (3) (*open symbols*). The absolute values obtained here agree remarkably well with those obtained on independently prepared actin samples using the mica plate instrument (Fig. 2), an instrument based on a totally different method for obtaining the dynamic moduli. We then repeated these measurements using ATP- and ADP-actin prepared using the hexokinase/glucose procedures (16; described in the methods section above) so that the ADP-actin samples would have minimal ATP contamination, again adding gelsolin at a ratio of 1:1,000 (*filled symbols*). The results from both procedures are very similar although the G′ and G″ values with properly prepared ADP-actin are somewhat higher. The filled symbols represent the average results from data on 7 separate actin preparations and 3 gelsolin preparations. It is noted that the shapes of the G′ and G″ curves are still very similar for F-ADP-actin compared with F-ATP-actin. Although the F-ADP-actin results are systematically higher than those of the ATP-actin by a factor of 2–3, these differences are just at the uncertainty limit of the measurement, based on the variability between samples. These differences are much smaller than those of Janmey et al. (3).

When solutions of F-ADP-actin (prepared by the hexokinase/glucose method) and F-ATP-actin were subjected to strain sweeps, at a constant frequency of 1 rad/s, the elastic modulus of both types of samples slowly decreased with increasing strain as shown in Fig. 4. Up to at least a 50% strain rate, the relative data for F-ADP- and F-ATP-actin agree, showing no difference in strain rate dependence of G′ for the two types of actin polymer. This agreement is in strong contrast to the data of Janmey et al. (3) who found that the F-ADP-actin data fell rapidly to zero at strain rates between 10 and 20%, while the F-ATP-actin data showed strain hardening below 10% strain, followed by a slower decrease up to the maximum strain rate studied of 25%. At strain rates above about 50%, we find that the F-ADP-actin data fall off somewhat faster with increasing strain rate. After strain sweeps (from low to high strain), the samples were monitored at 2% strain and the viscoelastic parameters were observed to slowly (over one hour) return to the values observed prior to the strain sweep (data not shown).

While performing the experiments shown in Fig. 3, it appeared that much of the variability in the data could be ascribed to differences in gelsolin activity. Data with better reproducibility were obtained when gelsolin activities from different preparations were matched using a simple actin polymerization assay. This suggested that polymer length might be responsible for the small rheological differences observed between F-ATP-actin and F-ADP-actin, with the F-ADP-actin polymer being longer (see Discussion, below). We further investigated this possibility by reducing the gelsolin concentration for one ATP-actin sample to produce longer F-ATP-actin filaments. The results are shown in Fig. 5. F-ATP-actin at 1:2,000 gelsolin:actin ratio displayed G′ values higher than those for F-ATP-actin at 1:1,000 gelsolin:actin ratio and similar to those for F-ADP-actin at 1:1,000 gelsolin:actin ratio. The G″ values obtained for F-ATP-actin at the 1:2,000 gelsolin:actin ratio are intermediate between those for F-ATP-actin and F-ADP-actin at 1:1,000 gelsolin over most of the frequency range; however, at the higher frequencies there are deviations, still

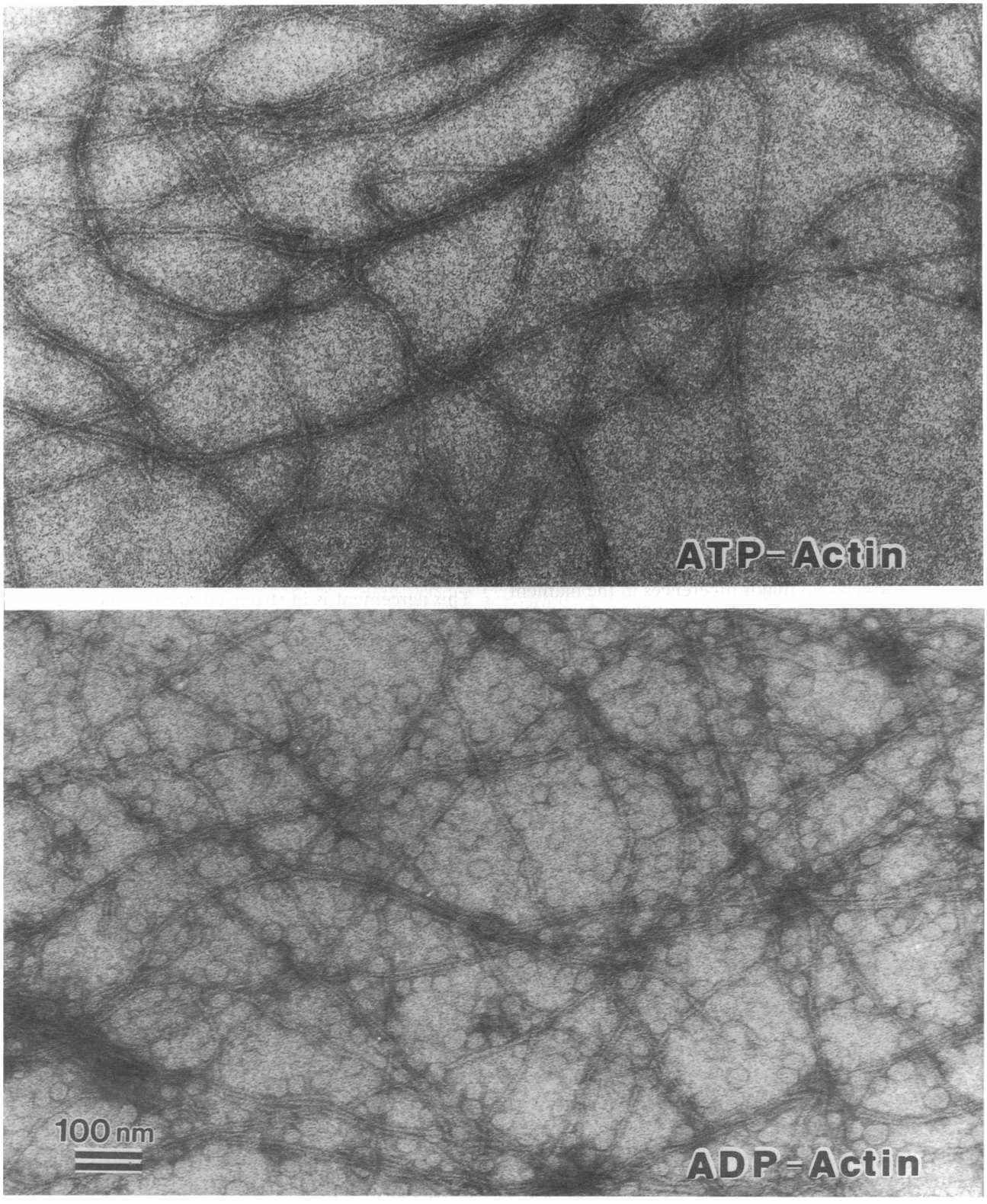

FIGURE 1 Representative fields showing F-actin prepared from ATP-actin monomer (*upper*) and ADP-actin monomer (*lower*). Magnification is 117,000×.

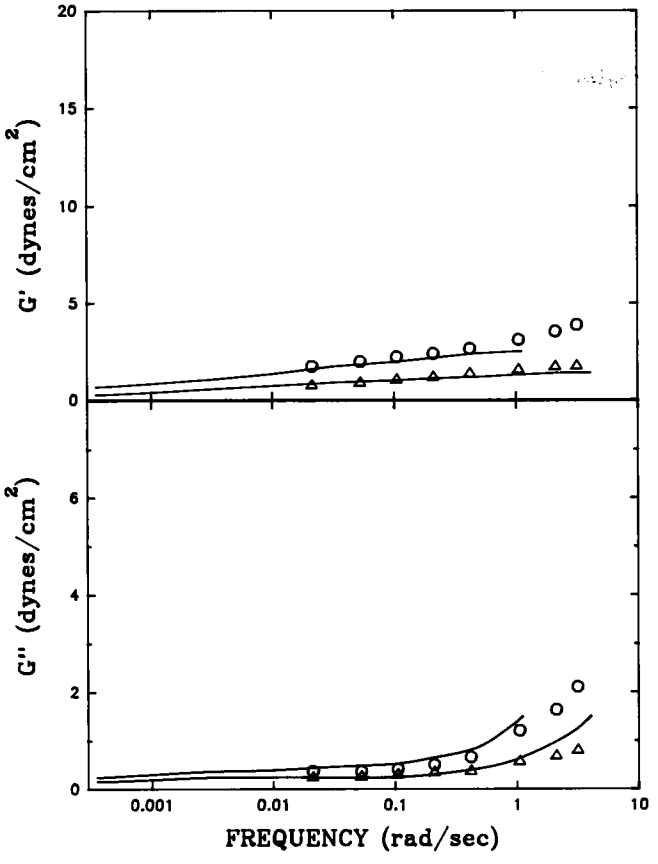

FIGURE 2 Dynamic storage, G', and dynamic loss moduli, G'', obtained using a mica-plate viscoelastometer, as functions of frequency for actin solutions, with gelsolin added at a ratio of 1:1,000. Actin was prepared essentially following the procedures of Janmey et al. (3), but adjusted to pH 7.4. The data points [(○) ADP-actin, (△) ATP-actin] represent individual measurements at the indicated frequencies, while the solid lines represent a Fourier transform of a compliance measurement which allows efficient measurement of the viscoelastic parameters at very low frequencies.

within the sample to sample uncertainty, which we can not explain. Samples without gelsolin showed G' values nearly 25 times higher than samples with 1:1,000 gelsolin and these samples reached plateaus only after very long times (data not shown).

Nucleotide exchange

The time course of the release of α^{32}P-labeled actin-bound nucleotide from F-ADP-actin and F-ATP-actin is shown in Fig. 6. A rapid 4–6% initial burst of release of α^{32}P-ADP was noted, similar to that observed earlier by others (11, 20–22). This has been attributed to bound nucleotide exchange on the monomer actin fraction in equilibrium with the polymer actin fraction. Release of the actin-bound α^{32}P-ADP occurred very slowly and at the same rate in the F-ATP-actin and F-ADP-actin samples over the 14-day time course. Clearly, the slow bound nucleotide exchange was independent of the type of monomer (ATP-actin or ADP-actin) which was used to form the two types of polymer actin. A recent study (12) of nucleotide exchange placed a lower limit on nucleotide exchange from actin polymer of 1,000 times slower than exchange from monomer, although the rate was too slow to be determined in their measurements.

DISCUSSION

While electron microscopy has been used to show gross ultrastructural differences between filaments prepared from ATP-actin monomer and ADP-actin monomer (3), we find no major differences. A recent careful study by Bremer et al. (23) finds small variations of the cross-over spacing and filament width which depend on the state of hydrolysis of the bound nucleotide. Our conclusion that there are no major differences between the differently prepared filaments is further supported by an-

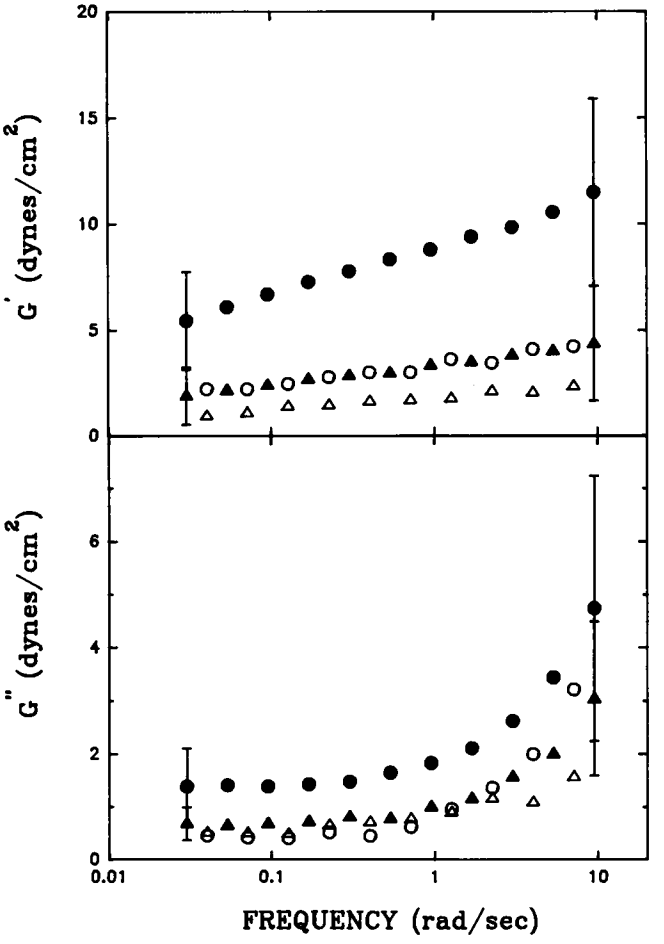

FIGURE 3 Data similar to those of Fig. 2, but with measurements made on a Rheometrics RFS II Fluids Spectrometer. The open symbols indicate actin samples, with added gelsolin at a ratio of 1:1,000, prepared following the exact published procedures of Janmey et al. (3; (○) ADP-actin, (△) ATP-actin). The filled symbols and associated error bars are for actin prepared as discussed in the Methods section using the hexokinase/glucose method to produce ADP-actin monomer and similar polymerization procedures ((●) F-ADP-actin, (▲) F-ATP-actin).

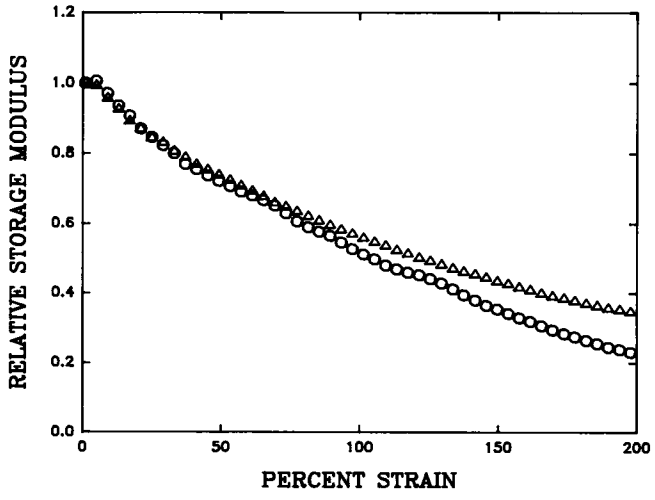

FIGURE 4 The strain dependence of the relative dynamic storage modulus, G', for F-ADP-actin (prepared using the hexokinase/glucose method) (○) and ATP-actin (△), both with gelsolin added at a ratio of 1:1,000 and obtained on the Rheometrics RFS II Fluids Spectrometer at a constant frequency of 1 rad/s. No strain hardening is observed nor is there a precipitous drop in the viscoelastic parameters which might suggest much filament breakage. The relative data for the two types of actin agree completely up to strains of about 50%.

other recent study (12) which finds ADP-actin filaments to have smooth profiles with no apparent flexibility differences from ATP-actin filaments.

A remarkable property of an F-actin solution, in view of the low mass concentrations present in the system, is that it has elastic or "gel-like" properties over a wide range of frequencies. This is indicated by the observation that the storage modulus, G', is greater than the loss modulus, G", for actin over a wide range of frequencies. An explanation of this phenomenon, consistent with contemporary models of polymer behavior, is that there is an entropically driven mechanism of rigidity, due to the fact that actin filaments are highly elongated and can impede each others' ability to rotate freely, and therefore the energy is stored in the configuration of the system as a whole. The elastic behavior of the system requires that the filaments be sufficiently extended to overlap in solution. In fact, conventionally prepared F-ATP-actin filaments are only slightly flexible (24, 25); for actin filament lengths less than 5 μm, the end-to-end length is over 95% of the contour length.

Clearly, an alteration of the flexibility of actin filaments would be expected to affect the observed mechanical properties of a solution of F-actin. In the limit of completely flexible filaments (ie., a random coil), the end-to-end lengths of the polymer coils is drastically reduced. For example, since the end-to-end distance is given by $l(n)^{1/2}$, where l is the length of a unit in the chain (2.7 nm for F-actin) and n is the number of links between units (about 370/μm for F-actin), the end-to-end distance of a 4 μm contour length polymer will be about 100 nm, which is probably less than the interfilament spacing at 35 μM actin. Therefore, in the extreme limit of filaments which are completely flexible, they will not overlap and the solution would not be expected to exhibit elastic behavior. Although the above example is a limiting case, rheometric measurements of G' and G" would be expected to be sensitive to the extent of overlap in the solution which is a function of the flexibility of the individual filaments and of their length distribution.

Rheological measurements reported here were performed in two different laboratories, using different actin preparations, and two entirely different instrumental approaches. All measurements show a similar dependence on frequency of the viscoelastic parameters for F-ATP-actin and F-ADP-actin with the F-ADP-actin parameters a factor of 2–3 larger in the presence of a 1:1,000 gelsolin:actin ratio. These results were consistently observed in both labs, using many different actin

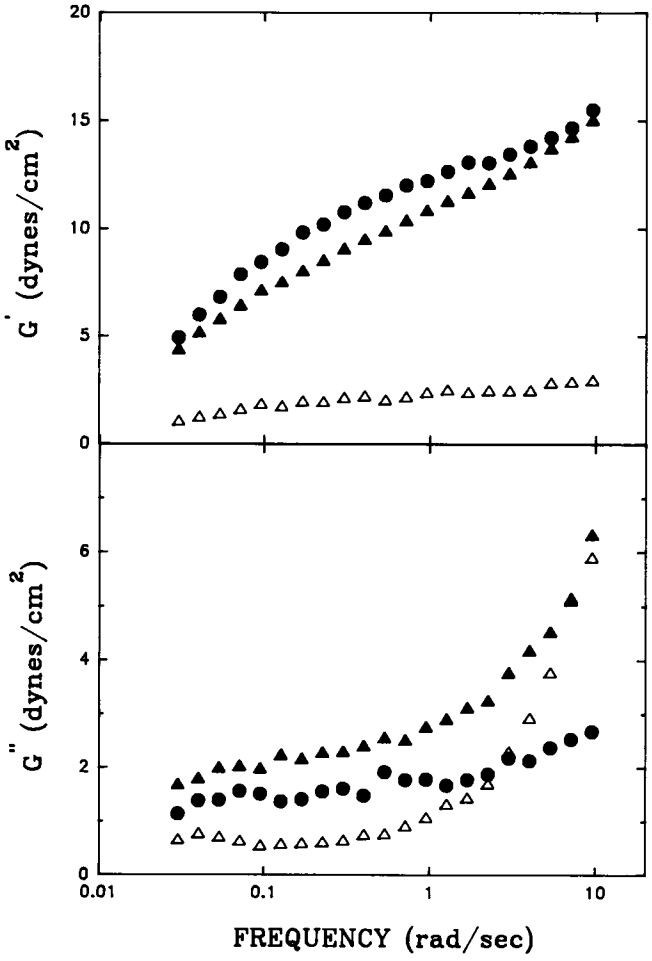

FIGURE 5 Data similar to those in Fig. 3, also measured on the RFS II instrument. The three sets of data shown represent: (△) F-ATP-actin with gelsolin added at a molar ratio of 1:1,000; (▲) F-ATP-actin with gelsolin added at a molar ratio of 1:2,000; (●) F-ADP-actin (prepared using the hexokinase/glucose procedure) with gelsolin added at a molar ratio of 1:1,000. Note that the data obtained from F-ATP-actin with gelsolin added at 1:2,000 are similar to the data for F-ADP-actin at 1:1,000 added gelsolin, particularly for G'.

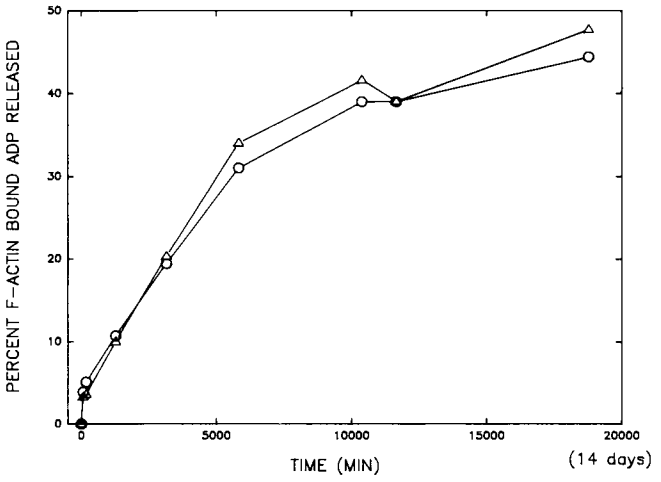

FIGURE 6 Exchange of ATP into 20 μM F-actin prepared from ADP-actin monomer (○) and ATP-actin monomer (△), as measured by α^{32}P-ADP release. The ADP-actin was prepared using the hexokinase and glucose method. Note the very slow exchange rate.

and gelsolin preparations, and are in sharp contrast to the results of Janmey et al. (3). Our viscoelastic parameter values for F-ATP-actin are in good agreement with those of other laboratories (8, 18, 22, 26–28), but are about 2 orders of magnitude smaller than those reported by Janmey et al. (3). It is not clear why these differences exist. A recent report by Pollard et al. (12), in which G′ and the dynamic viscosity are reported for ADP- and ATP-actin, without added gelsolin, indicates no difference in mechanical properties of the two actin filament types and G′ values slightly above our values for actin solutions with gelsolin present, as one might expect.

Central to the report by Janmey et al. (3) were experiments which showed a rapid incorporation of exogenous ATP into F-ADP-actin and a rapid straightening of F-ADP-actin filaments upon dilution into ATP-containing buffer. We find that the exchange of nucleotide into F-actin is very slow and independent of the bound nucleotide on the monomer from which the polymer was prepared. Such slow exchange is in agreement with earlier studies of nucleotide exchange into actin polymer (10, 29). The nucleotide exchange experiments reported by Janmey et al. (3) used 20 hours as an endpoint for completion of the exchange of ATP for ADP in a fluorescence assay. Our data (see Fig. 6) suggest that less than 10% exchange has occurred in this time and thus, we suspect that their analysis considered nucleotide exchange on only 10% of the actin. This might explain their observations, since a significant portion of the first 10% of the ADP-actin which exchanges will be monomer which then polymerizes to the critical concentration of ATP-actin (12). The half-time for exchange of ADP for ATP on monomer actin has been shown to be on the order of 1,000 sec (12, 30, 31), similar to the exchange rate reported by Janmey et al. for polymer actin. The exchange of exogenous ATP into actin polymer is clearly much slower as shown here and as recently pointed out by Pollard et al. (12), and is much too slow to cause "reversal" of ADP-actin polymer to ATP-actin polymer in the time frame reported by Janmey et al.

The dialysis method of preparing ADP-actin outlined by Janmey et al. (3) results in high residual levels (up to 60%) of ATP-actin. Thus, the F-ADP-actin studies reported by these workers represented measurements on very mixed populations of filaments. The hexokinase/glucose method was most effective in the preparation of ADP-actin provided the actin was converted to Mg-actin as previously reported (16). In attempting to reproduce the rheology data of Janmey et al., we prepared ADP-actin following both procedures.

The small systematic differences observed between viscoelastic parameters for F-ADP-actin and F-ATP-actin are conceivably due to very small flexibility differences, but are more probably due to length differences in the actin preparations. We initially found that the differences were larger for different gelsolin preparations, before calibration of the gelsolin activity which led to more reproducible length distributions. Our results suggest that the action of gelsolin may be different during polymerization of ADP-actin and ATP-actin, perhaps due to the different nucleation characteristics of the two different actins. When performing rheological measurements on F-ATP-actin samples without added gelsolin, the incubation times required after the addition of salt in order to reach a steady-state are on the order of 12–24 hours. With added gelsolin, the required incubation times were about 1 hour for F-ATP-actin and roughly twice that time for F-ADP-actin samples under otherwise identical conditions. Given that the mean filament lengths are much longer in the absence of gelsolin than in the presence of a 1:1,000 ratio of gelsolin to actin, the longer time for F-ADP-actin to reach steady-state rheological parameters also suggests that the F-ADP-actin filaments may be somewhat longer than the F-ATP-actin filaments. This would also perhaps explain the slightly greater decrease in relative elastic modulus of F-ADP-actin than F-ATP-actin at very high strain rates where filament breakage would preferentially affect the G′ contribution of longer filaments. The effects of ATP vs ADP on gelsolin activity are also not well understood at present. A recent report (32) indicates that ATP binds directly to gelsolin and thus the nucleotide present may have direct effects on gelsolin activity. We are currently further investigating this as a possible basis for the small residual differences in viscoelastic parameters between F-ADP-actin and F-ATP-actin.

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