Evidence that F-actin Can Hydrolyze ATP Independent of Monomer-Polymer End Interactions*

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The rate of ATP hydrolysis in solutions of F-actin at steady state in 50 mM KCl, 0.1 mM CaCl₂ was inhibited by AMP and ADP. The inhibition was competitive with ATP (Kᵢ = 6.0 μM) with Ki values of 9 μM for AMP and 44 μM for ADP. ATP hydrolysis was inhibited >95% by 1 mM AMP. AMP had no effect on the time course of actin polymerization, ATP hydrolysis during polymerization, or the critical actin concentration. Simultaneous measurements of G-actin/F-actin subunit exchange and nucleotide exchange showed that nucleotide exchange occurred much more rapidly than subunit exchange; during the experiment over 50% of the F-actin-bound nucleotide was replaced when less than 1% of the F-actin subunits had exchanged. When AMP was present it was incorporated into the polymer, preventing incorporation of ADP from ATP in solution. F-actin with bound Mg²⁺ was much less sensitive to AMP than F-actin with bound Ca²⁺. These data provide evidence for an ATP hydrolysis cycle associated with direct exchange of F-actin-bound ADP for ATP free in solution independent of monomer-polymer end interactions. This exchange and hydrolysis of nucleotide may be enhanced when Ca²⁺ is bound to the F-actin protomers.

Monomeric actin has a single high affinity nucleotide binding site which binds ATP about 175 times more tightly than ADP (1). Actin polymerization in the presence of ATP is accompanied by hydrolysis of the actin-bound ATP to produce polymeric actin containing predominantly bound ADP. Early evidence, however, showed that ATP hydrolysis was not obligatory for monomer addition since monomeric actin with bound ADP (2, 3) or adenyl-5'-yl-imidodiphosphate (3), a nonhydrolyzable analogue of ATP, was polymerizable. Furthermore, the polymerization of G-actin-ATP was unaffected by the addition of Pₐ, a product of the hydrolysis reaction, supporting the idea that ATP hydrolysis and monomer addition were probably not coincident events (3).

Recently, Fardee and Spudich (4) found that under certain conditions ATP hydrolysis can lag significantly behind F-actin formation, and Mockrin and Korn (5) demonstrated that polymerization of chemically cross-linked actin dimer occurs much faster than hydrolysis of dimer-bound ATP. Therefore, a minimal scheme for ATP hydrolysis during actin polymerization requires at least the transient existence of F-actin with bound ATP, i.e. monomeric actin-ATP adds to the filament end and hydrolysis of ATP occurs on the filament after the addition reaction (6, 7).

When polymerization is complete, ATP hydrolysis continues at a constant steady state rate (8) and a slow exchange of F-actin-bound ADP for ADP derived from free ATP has been demonstrated (9). It has been generally held (8) that the ATP hydrolysis at steady state results primarily from the cyclic addition and loss of actin monomers from filament ends with concomitant hydrolysis of monomeric actin-bound ATP. When F-actin solutions are subject to ultrasonic vibration, the rate of ATP hydrolysis increases, returning to its original value only gradually after sonication is ended (8, 10). These observations are consistent with the breakage of long filaments by the vibration, producing a solution containing many more polymer ends with which monomers can interact, followed by a slow annealing and loss of ends as the solution returns to its steady state polymer length distribution.

Recent measurements by Brenner and Korn (11) of the rates of monomer-polymer subunit exchange and steady state ATP hydrolysis in F-actin solutions have led to the new hypothesis that at least one end of an F-actin filament may have a segment of actin-ATP protomers as steady state and well as during pre-steady state polymerization. ATP hydrolysis coupled with monomer-polymer end interaction would then occur only when the length of this actin-ATP cap increased beyond some maximal permissible size resulting in hydrolysis of the F-actin-bound ATP most distal to the filament end. In this scheme, the (equal) rates of monomer addition and loss from filament ends at steady state could be much faster than the measured ATP hydrolysis rate, because actin-ATP subunits could exchange without hydrolysis of ATP, while monomer addition and ATP hydrolysis could be more closely coupled during polymerization.

In this study, we have examined the effects of several nucleotides on the rate of ATP hydrolysis in solutions of F-actin at steady state and have found AMP to be a particularly effective inhibitor. The results show that under some ionic conditions ATP hydrolysis is predominantly associated with direct exchange of ATP in solution for ADP in the F-actin structure.

MATERIALS AND METHODS

N-Pyrenlidoacetamide from Molecular Probes was used without further purification. ATP was from Sigma. ADP, AMP, and UMP were from P-L Biochemicals. [γ-³²P]ATP and [α-³²P]ATP from New England Nuclear were diluted immediately upon arrival to 0.5-1.0 mCi/ml in 2 mM ATP and stored at -20 °C. [³²P]AMP was from Amersham Corp. Procedures to determine the radiochemical purity and nucleotide concentrations were previously described (12). Dowex 1-X8 (200-400 mesh) was from Bio-Rad, and cytochalasin D was from Aldrich. Stock solutions of cytochalasin D were prepared in dimethyl sulfoxide.

Muscle G-actin was prepared from acetone powders of rabbit back and leg muscle by the method of Spudich and Watt (13), as modified

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by Eisenberg and Kielley (14), followed by gel filtration on Sephadex G-200. Monomeric actin was stored on ice in a buffer containing 5 mM Tris HCl, 0.2 mM dithiothreitol, 0.2 mM ATP, 0.1 mM CaCl₂, and 0.01% NaN₃, pH 8.0 (buffer G). The concentration of monomeric actin was determined either from its absorbance at 290 nm, using an extinction coefficient of 0.617 mg⁻¹ cm⁻¹ (15), by the procedure of Lowry et al. (16) or the procedure of Bradford (17). In the latter two cases muscle G-actin was used as a standard.

G-actin was labeled with [γ³²P]ATP, and the bound nucleotide (0.95–1.03 mol of ATP/mol of actin) and total exchangeable nucleotide (typically >0.95 mol of ATP/mol of actin) were determined as previously described (18). Hydrolysis of [γ³²P]ATP was followed by measuring the release of ³²P (19).

Pyrenyl actin¹ was prepared by reacting actin with N-pyrenylidooacetamide using the procedure of Koyama and Mihashi (20) with minor modifications (11). To avoid bleaching the fluorophore, pyrenyl actin was prepared and stored in the dark and, during fluorescence measurements, was exposed to the light source only intermittently.

Polymerization and steady state monomer-polymer subunit exchange measurements were made using an SLM 4000 or Spex Photomolog 2 spectrofluorimeter with the sample chamber maintained at 30 °C. All measurements were made on samples in quartz cuvettes (3 × 10 mm in 4 mM MgCl₂) with the long dimension of the cell parallel to the incoming light path. For 90° light scattering experiments, the excitation and emission wavelengths were 450 nm and the excitation and emission bandwidths were 2 nm. For fluorescence measurements on pyrenyl actin, the excitation and emission wavelengths were 368 and 388 nm (98° and 386 nm on the Spex instrument) and the resonant scattering settings were 1 and 4 nm (0.9 and 4.5 mm on the Spex instrument).

Monomer-polymer subunit exchange measurements were performed as previously described (11). Briefly, a trace amount of pyrenyl G-actin (typically <10% of the critical concentration) was added to a solution of F-actin at steady state and the time course of any fluorescence increase was observed. A separate sample of pyrenyl G-actin was copolymerized with G-actin to determine the equilibrium fluorescence.

Critical concentrations were determined by polymerizing actin (20 μM, 5% pyrenyl actin, 1 ml) at 30 °C, sonicating on ice for 5 s at setting 3 on a Heat Systems W185 Ultrasonic Sonifier equipped with micropipet, and rapidly diluting to multiple lower actin concentrations. The fluorescence of each sample was measured after a 1.5-h incubation at 30 °C. The observed fluorescence values did not significantly change upon longer incubation (16–20 h).

Dowex 1-X8 (50% by volume in H₂O) was equilibrated by four washes with a 2-fold excess of buffer G prepared without ATP and containing either 50 mM KCl, 4 mM MgCl₂, or 2 mM MgCl₂. The final Dowex suspensions were made 50% by volume in the respective buffers and stored at 5 °C.

The nucleotide exchange on F-actin, unbound nucleotide was first removed from F-actin (24 μM) at steady state in 50 mM KCl. Dowex in 50 mM KCl was added to the actin (20% by volume, 2.4 ml/12 ml of actin solution), the solution mixed gently by inversion, and the Dowex removed by low speed centrifugation. The procedure was then repeated. Greater than 99% of the free nucleotide was removed by this protocol.

Aliquots of Dowex-treated F-actin (2 ml) were then added to glass tubes (13 × 100 mm) containing various radiolabeled nucleotides (see "Results," Fig. 5C) and the samples were incubated at 30 °C. At various times 0.2-ml aliquots were removed and added to 0.2 ml of Dowex in 4 mM MgCl₂. After a 30-s incubation, the resin was removed by centrifugation, and 0.2 ml of the supernatant was added to 0.2 ml of Dowex in 2 mM MgCl₂. The final supernatant was collected and spun again to remove any traces of resin. Both the protein concentration and radioactivity in the final supernatant were determined. This procedure removed >99.5% of the unbound nucleotide. Dilution into KCl-free Dowex solutions enhanced the binding of free nucleotide by the high MgCl₂ concentration, protecting the actin against depolymerization.

RESULTS

Effects of AMP, ADP, and P on ATP Hydrolysis in Solutions of F-actin at Steady State—To investigate the effect of the products of ATP hydrolysis and related nucleotides on the steady state ATP hydrolysis rate, F-actin was prepared in the presence of 0.26 mM [γ³²P]ATP. The rate of ATP hydrolysis was measured at 25 °C (Table I). At a concentration of 50 μM, P, had no effect on the rate of hydrolysis of ATP. At the same concentration, ADP and AMP inhibited the rate of ATP hydrolysis by about 40 and 70%, respectively. Neither UMP nor GMP at concentrations as high as 1 mM had any effect. Since AMP appeared to be a potent inhibitor of ATP hydrolysis by F-actin at steady state, its effects on actin polymerization were examined in more detail.

Actin Polymerization and Associated ATP Hydrolysis—G-actin was equilibrated in buffer G containing 0.29 mM ATP and polymerization was initiated at 30 °C by the addition of 50 mM KCl. Polymerization (Fig. 1A) and associated ATP hydrolysis (Fig. 1B) were monitored as described above. Polymerization was complete in about 60 min at which time about 0.94 mol of ATP had been hydrolyzed per mol of total actin. Under these conditions, hydrolysis of actin-bound ATP was tightly coupled to polymerization as previously reported (11). After polymerization was complete, hydrolysis of ATP continued at a steady state rate of about 0.4 mol of ATP/mol of total actin/h (Fig. 1B, Q). This concentration of 50 μM AMP had no effect on the time course of actin polymerization (Fig. 1A) or on the hydrolysis of ATP during polymerization (Fig. 1B, Q). This concentration of AMP, however, inhibited the steady state ATP hydrolysis rate by about 65%, to about 0.13 mol of ATP/mol of actin/h.

The same extent of inhibition was observed whether the AMP was added to G-actin before polymerization or to a solution of F-actin already assembled to steady state. At similar concentrations neither ADP nor P had any effect on the time course of polymerization or ATP hydrolysis during polymerization.

The extent of inhibition was examined as a function of the concentration of AMP (Fig. 2). Nearly complete (>95%) inhibition of ATP hydrolysis at steady state was observed at 1 mM AMP while concentrations of less than 1 mM AMP had no detectable effect. Half-maximal inhibition was observed at about 60 μM AMP for 25 μM actin in 0.25 mM ATP. In order to minimize any ionic strength changes in this experiment, the total added nucleotide was kept constant at 1 mM by addition of UMP, which alone had no effect. Essentially the same inhibition curve was obtained if UMP was omitted.

Since steady state ATP hydrolysis in solutions of F-actin has been thought to result either directly or indirectly from the continued addition and loss of monomers from filament ends, it seemed likely that the observed decrease in ATPase rate might be the result of a decrease in the critical concentration of G-actin coexisting with polymeric actin at steady state. As shown in Fig. 3, however, 500 μM AMP had no effect.

![Table I](http://www.jbc.org/)

<table>
<thead>
<tr>
<th>Addition</th>
<th>ATP hydrolysis rate (mol ATP/mol actin/h)</th>
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<tr>
<td>None</td>
<td>0.22</td>
</tr>
<tr>
<td>50 μM P</td>
<td>0.22</td>
</tr>
<tr>
<td>50 μM ADP</td>
<td>0.13</td>
</tr>
<tr>
<td>50 μM AMP</td>
<td>0.06</td>
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¹ The trivial name and abbreviation used are: pyrenyl actin, N-pyrenylcarboxyoxamidomethyl actin; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid.

**TABLE I**

AMP and ADP inhibit ATP hydrolysis in F-actin solutions at steady state

F-actin (120 μM) in buffer G containing 30 mM KCl was diluted to 24 μM in the same buffer containing 3.260 μM [γ³²P]ATP. After a 30-min preincubation at 25 °C to establish a steady state, the additions listed below were made to aliquots of the F-actin solution. ATP hydrolysis was monitored and was linear with time for at least 150 min.
ATP Hydrolysis by F-actin

Fig. 1. Effect of AMP on actin polymerization and associated ATP hydrolysis in 50 mM KCl. G-actin (22 µM) equilibrated in buffer G containing 0.29 mM [γ-32P]ATP was polymerized at 30 °C by addition of 50 mM KCl. A, polymerization was followed by light scattering. B, simultaneous measurements of ATP hydrolysis were made on a parallel sample. Actin was polymerized in the presence (dashed lines, ●) or absence (solid lines, ○) of 50 µM AMP.

Fig. 2. AMP inhibition of steady state ATP hydrolysis in solutions of F-actin. G-actin (25 µM) equilibrated in buffer G containing 0.25 mM [γ-32P]ATP was polymerized for 1.5 h at 30 °C by addition of 50 mM KCl. Various concentrations of AMP were added to aliquots of the F-actin. ATP hydrolysis was monitored and was linear for at least 1.5 h. In this experiment, the added AMP was supplemented with UMP to keep the total added nucleotide at 1 mM in all cases. UMP alone had no effect on the steady state ATP hydrolysis rate.

Fig. 3. Effect of AMP on the actin critical concentration in 50 mM KCl. F-actin (20 µM, 5% pyrenyl actin) in buffer G containing 50 mM KCl was sonicated for 5 s and diluted to multiple actin concentrations in the same buffer in the presence (●) or absence (○) of 0.5 mM AMP. After incubation at 30 °C for 1.5 h, the final fluorescence was determined. The critical concentration of 0.34 µM was unaffected by AMP. The critical concentration varied from 0.34–0.5 µM for various actin preparations.

No evidence for the binding of AMP to G-actin was found using equilibrium dialysis, either with actin in buffer G or with actin in buffer G + 50 mM KCl below the critical actin concentration (data not shown). Therefore, the effect of AMP on the rate of ATP hydrolysis at steady state is not the result of a change in either the size or the nucleotide content of the G-actin pool.

No detectable ATP hydrolysis was measured for monomeric actin below its critical concentration in 50 mM KCl either in the presence or absence of AMP. This is in agreement with our earlier observation that Mg2+ is required for the expression of monomeric actin ATPase activity (21).

AMP and ADP Compete with ATP for Sites of ATP Hydrolysis in Steady State F-actin Solutions—Samples of F-actin in 50 mM KCl containing a range of concentrations of ATP were prepared and the rate of steady state ATP hydrolysis was measured in the absence of any additions (Fig. 4, A and B, ●), in the presence of 10 µM AMP (Fig. 4A, ○), or in the presence of 50 µM ADP (Fig. 4B, ○). Double reciprocal plots of the steady state ATP hydrolysis rate as a function of ATP concentration were linear both in the presence and absence of inhibitors. The inhibitors had no effect on Vmax for the ATP hydrolysis reaction, but only changed Kcat indicating that ADP and AMP are competitive inhibitors, competing with ATP for the site of nucleotide hydrolysis. At 25 °C, the Kcat values for ATP in the two experiments were 530 µM (Fig. 4A) and 667 µM (Fig. 4B), and the Vmax values were 0.63 and 1.1 mol of ATP hydrolyzed per mol of actin/h. The reason for the variation in Vmax is not known.

The inhibitor constants K, calculated from the data in Fig.
ATP Hydrolysis by F-actin

Fig. 4. Lineweaver-Burk analysis of inhibition of F-actin ATPase by AMP and ADP. [γ-32P]ATP was added to multiple samples of F-actin in 50 mM KC1 at 25 °C to give final ATP concentrations in the range 0.1–1 mM. The final actin concentrations were 24 μM (A) or 21 μM (B). Samples were prepared containing 10 μM AMP (A, O), 50 μM ADP (B, O) or neither (O). After incubation at 25 °C for 30 min the rate of ATP hydrolysis was monitored and was linear with time for at least 4 h. The ATPase rate is calculated as moles of Pi produced per mol of actin/h.

AMP had no effect, however, on the kinetics of monomer-polymer subunit exchange (Fig. 5B) monitored by adding a trace of pyrenyl G-actin to F-actin at steady state. Initially, all of the fluorescent monomers were in the G-actin pool. Complete randomization of the label between the G- and F-actin pools would have resulted in the fluorescent signal indicated by the dashed line in Fig. 5B. After an initial burst, the fluorescence data indicated essentially no further exchange of subunits.

It is important to realize that the fluorescence is not a linear function of the amount of F-actin that had exchanged (11). For example, if the critical concentration were small compared to the total actin concentration (as it is in this case) only a fraction of the F-actin subunits need exchange to incorporate

4 are 9 μM for AMP and 44 μM for ADP. These K values represent true dissociation constants of AMP and ADP at the ATP hydrolysis site on F-actin. AMP is a more effective inhibitor of steady state ATP hydrolysis than ADP because it binds more tightly to the “active site” on F-actin.

ATP Hydrolysis, Actin Monomer-Polymer Subunit Exchange, and Nucleotide Exchange at Steady State—To characterize further the ATP hydrolysis reaction in solutions of F-actin at steady state, simultaneous measurements were made of ATP hydrolysis, monomer-polymer subunit exchange, and nucleotide exchange in the presence and absence of 100 μM AMP (Fig. 5) at 30 °C. As shown in Fig. 5A, in this experiment AMP decreased the initial rate of ATP hydrolysis from 1.1 to 0.17 mol of ATP/mol of actin/h.

Fig. 5. Effects of 100 μM AMP on ATP hydrolysis, monomer-polymer subunit exchange, and nucleotide exchange of F-actin in 50 mM KC1 at 30 °C. Unbound nucleotide was removed from F-actin in buffer G + 50 mM KC1 using Dowex 1-X8 as described under “Materials and Methods.” Aliquots of Dowex-treated F-actin (15 μM) were analyzed as follows. A, to one sample, [γ-32P]ATP (204 μM) was added and ATP hydrolysis was monitored in the presence (O) or absence (●) of 100 μM AMP. B, pyrenyl G-actin (0.04 μM) was added to each of the solutions prepared in (A) and monomer-polymer subunit exchange was followed by monitoring the increase in fluorescence as described under “Materials and Methods.” A sample of G-actin was copolymerized with pyrenyl G-actin to determine the fluorescence at equilibrium (dashed lines). C, to measure ADP exchange into F-actin, samples were prepared containing [α-32P]ATP with (O) or without (●) 100 μM AMP. To measure AMP incorporation into actin, [32P]AMP was added to one sample (O) in the presence of unlabeled ATP (204 μM). At the times indicated, aliquots of each solution were analyzed to determine the amount of bound labeled nucleotide as described under “Materials and Methods.”
most of the labeled G-actin into polymer. The amount of F-actin exchanged, X, at time t can be calculated from the zero time fluorescence, \( F(0) \), the equilibrium fluorescence, \( F_e(\infty) \), the critical actin concentration \( A_c \), and the fluorescence at time t, \( F(t) \), using the equation (see derivation in Ref. (11))

\[
\frac{F(t) - F(0)}{F_e(\infty) - F(0)} = 1 - \exp(-X/A_c)
\]

From the data in Fig. 5B, we find that the maximum amount of actin subunit exchange, either in the presence or absence of AMP, was about 0.14 \( \mu M \) or about 1% of the total F-actin present. Under these ionic conditions, therefore, almost no exchange of monomer subunits with polymer subunits occurs during a 4-h incubation.

On the other hand, Fig. 5C shows that substantial nucleotide exchange occurs in the absence of actin subunit exchange. When \( [\alpha^{32P}]ATP \) was added to a solution of F-actin at steady state, radioactivity was bound to F-actin at an initial rate of about 0.1 moles of labeled nucleotide/mol of actin (note that the critical concentration represented only about 2% of the total actin so that any labeled nucleotide bound to actin above that level must have been into F-actin). This nucleotide incorporation rate is about one-tenth the ATP hydrolysis rate seen in the same solution (Fig. 5A). We assume that the nucleotide incorporated into polymer was \([\alpha^{32P}]ADP\) generated from labeled ATP, since significant concentrations of F-actin-bound ATP at steady state have never been reported. Thus, in the first 4 h, F-actin had exchanged greater than 30% of its bound nucleotide under conditions where less than 1% of its subunits had exchanged with G-actin in the monomer pool. Apparently, in 50 mM KCl, nucleotide exchange occurs directly between the solution and polymer-bound nucleotide and is not mediated by actin subunit exchange.

AMP strongly inhibited the incorporation of labeled ADP from ATP into F-actin (Fig. 5C, O). Using \([32P]AMP\) it was found that under these conditions AMP was incorporated into F-actin (Fig. 5C, □). After incubation for 7.5 h nearly 40% of the F-actin subunits contained bound AMP. The total nucleotide incorporated when AMP was present (i.e., AMP plus ADP from ATP) was approximately the same as the amount of ADP incorporated from ATP when AMP was absent.

**Ca-Actin and Mg-Actin Exhibit Differential Sensitivities to AMP—** Preliminary experiments indicated that the steady state ATP hydrolysis rate for actin polymerized in 2 mM MgCl₂ was not affected by addition of 50 \( \mu M \) AMP. Three possible explanations for this observation are that i) only actin polymerized in the presence of KCl is sensitive to AMP, ii) actin polymers formed from actin monomers containing bound Ca²⁺ are sensitive to AMP while polymers containing Mg-protons are insensitive, or iii) the differential sensitivity arises from the differences in the inhibitors and substrates, i.e., CaAMP versus MgAMP and CaATP versus MgATP.

The experiments described in Table II suggest that it is a difference between Ca-F-actin and Mg-F-actin that results in the differential sensitivity of the steady state ATPase to AMP. When G-actin in 0.1 mM CaCl₂ was polymerized in 50 mM KCl, the steady state ATPase rate was inhibited 47% by 50 \( \mu M \) AMP (Table II, Experiment 1). When the same G-actin was first incubated in 0.5 mM EGTA, 0.1 mM MgCl₂ to replace the bound Ca²⁺ with Mg²⁺, and then polymerized with 50 mM KCl, the steady state ATP hydrolysis rate was unaffected by 50 \( \mu M \) AMP. This indicates that the differential sensitivity did not involve the presence or absence of KCl.

To attempt to distinguish between possibilities (ii) and (iii) F-actin prepared from Ca- or Mg-monomers was polymerized in 50 mM KCl and then 0.5 mM EDTA was added to chelate excess divalent cations. The steady state ATP hydrolysis rates measured after addition of EDTA were linear with time for several hours and the amount of polymer in solution remained constant as assayed by light scattering, indicating that little, if any, denaturation was associated with EDTA addition. The ATP hydrolysis rates were measured in the presence and absence of several AMP concentrations (Table II, Experiments 2 and 3). The ATPase activity of polymers containing Ca²⁺ showed a pronounced sensitivity to AMP while Mg-polymer were much less sensitive with or without addition of EDTA. The addition of EDTA increased the ATP hydrolysis rate for Ca-F-actin but had little, if any, effect on Mg-F-actin. The ATPase activity of Mg-F-actin was inhibited slightly by 200 \( \mu M \) AMP. Other data (not shown) indicate that the Kₛₐₐ for ATP of Mg-F-actin is approximately 40 \( \mu M \) or about one-tenth the value for Ca-F-actin. It remains to be determined if the differential sensitivity of Ca-F-actin and Mg-F-actin to AMP is explained solely by this difference in Kₛₐₐ or if the AMP-binding constants (Kₐ) are also different for the two polymers.

As shown in Table II, Experiment 1, 50 \( \mu M \) AMP inhibited ATP hydrolysis by Ca-F-actin even when 1 \( \mu M \) cytochalasin D was present. Cytochalasin D alone had no effect on the ATP hydrolysis rate for Ca-actin but stimulated the rate over 2-fold for Mg-actin, presumably because of stimulating the ATPase activity of Mg-G-actin monomer (21). The ATP hydrolysis rate of monomeric actin below the critical actin concentration in buffers containing Mg²⁺ (6) was unaffected by addition of AMP (data not shown).

**DISCUSSION**

The observations presented in this paper imply that ATP hydrolysis in solutions of F-actin at steady state can occur by a mechanism distinct from that associated with monomer-polymer end interactions. While it inhibited steady state ATP...
hydrolysis. AMP had no effect on the rate constants for nucleation, elongation, or dissociation of F-actin as evidenced by its lack of effect on the polymerization time course (which depends on the product of the elongation and dissociation rate constants) and the actin critical concentration (which depends on the ratio of the dissociation and elongation rate constants). Also, cytochalasin D, which is known to cap the barbed end of actin filaments (for a review, see Ref. (22)), had no measureable effect on the inhibition of ATP hydrolysis by AMP, further suggesting a lack of involvement of filament ends. On the other hand, ATP hydrolysis during polymerization proceeded normally even at AMP concentrations sufficient to abolish >95% of the steady state ATP hydrolysis rate so that AMP does not interfere with monomer-polymer end interactions.

The inhibition of ATP hydrolysis by AMP in the absence of any effect on the critical actin concentration suggests that either the subunit association and dissociation reactions that determine the critical concentration do not involve hydrolysis of ATP or that only a small, AMP-insensitive, fraction of the ATP hydrolysis events at steady state is associated with monomer-polymer end interactions. The former conclusion is consistent with the presence of an actin-ATP cap at the end of an actin filament such that the on- and off-reactions at steady state only rarely result in hydrolysis of actin-bound ATP (11).

Nucleotide exchange can occur independently of actin monomer exchange. Presumably, nucleotide exchange occurs from a “breathing” or “loosening” of the actin structure allowing exchange of F-actin-bound ADP for ATP in solution with associated hydrolysis. Such a mechanism was suggested previously to account for ATP hydrolysis during sonication of solutions of F-actin (23). The steady state ATP hydrolysis rate in the absence of AMP was about 10-fold higher than the measured nucleotide exchange rate, suggesting that when a breathing event occurs multiple hydrolytic cycles occur before the break in the polymer structure heals and the nucleotide is locked in.

AMP appears to inhibit the steady state ATP hydrolysis rate by exchanging with F-actin-ADP and binding with high affinity to the F-actin nucleotide site. The observation that the total nucleotide exchanged into F-actin is about the same in the presence or absence of AMP is consistent with this mechanism of inhibition. AMP is a more effective inhibitor than ADP, indicating that AMP binds more tightly than ADP to the F-actin nucleotide site. The replacement of bound ADP by AMP of sonication of F-actin in the presence of AMP has previously been reported (24), and our results show that AMP will replace ADP in F-actin even without external sonic vibration.

The $K_a$ value for ATP hydrolysis by F-actin in 50 mM KCl, 0.1 mM CaCl$_2$ is quite high (approximately 0.5 mM ATP) indicating that under commonly used conditions (0.2 mM ATP) the maximal rate of ATP hydrolysis would not be observed. In future studies on actin-catalyzed ATP hydrolysis it will be important to know the $V_{max}$ and $K_a$ values under the specific ionic conditions in order to make valid comparisons. Possibly, the reason the addition of EDTA to steady state F-actin solutions in 50 mM KCl, 0.1 mM CaCl$_2$, 0.2 mM ATP increased the rate of ATP hydrolysis was that the concentration of free substrate was increased (i.e. CaATP was replaced by uncomplexed ATP). Since the concentration of total ATP was less than the $K_a$, this would result in an increase in the ATP hydrolysis rate if ATP, and not CaATP, were the substrate. From other data, ATP and divalent cations may bind independently to F-actin. The measured affinities for ATP and divalent cations on G-actin are different ($K_a$ of 10$^{-10}$ versus 10$^{-5}$ M) (1) and the sites can be separated by proteolytic cleavage (25), although recent $^{31}$P NMR measurements suggest the two sites may be quite close together (26). The simplest interpretation of our data is that free ATP is the substrate for the ATP hydrolysis reaction which is inhibited by AMP.

Hydrolysis of ATP in actin solutions is a complex process which may occur independently on the monomer, on the polymer (either on an end or internal subunit), or indirectly coupled to monomer addition to polymer. It will be interesting to test the effects of various actin binding proteins on ATP hydrolysis by actin but results of such studies must be approached with caution unless comparisons are made under a defined set of ionic conditions and care is taken to determine what molecular mechanism is being affected.

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