F-actin-like ATPase Activity in a Polymerization-defective Mutant Yeast Actin (V266G/L267G)*

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Polymerization increases a low level G-actin ATPase activity yielding ADP-P, F-actin and then ADP F-actin following release of P. By monitoring P release, we explored the relationship between the ATPase activity and polymerization characteristics of a mutant yeast actin, GG. In this mutant, two hydrophobic residues at the tip of a proposed hydrophobic plug between actin subdomains 3 and 4, Val266 and Leu267, were mutated to Gly. Although GG-actin does not polymerize by itself in vitro, GG cells are viable. We show that GG-actin ATPase activity increases under normal polymerization conditions, although stable filaments do not form. A plot of P release rate versus actin concentration yields an apparent critical concentration, like that seen for actin polymerization, of ~8 μM for Mg2+ GG-actin and 11 μM for Ca2+ GG-actin. In contrast to WT-actin, P release from GG-actin is cold-sensitive, reflecting the temperature sensitivity associated with mutations that decrease hydrophobicity in this region. Thus, under polymerization conditions, GG-actin exhibits a continuous F-actin-like ATPase activity resulting from the temperature-sensitive formation of unstable cycling F-actin oligomers. Tropomyosin limits the extent and rate of this activity and restores polymerization by capturing and stabilizing these oligomers rather than enhancing filament nucleation.

G-actin, in the presence of Mg2+, slowly catalyzes the hydrolysis of ATP that is bound at a high affinity site at the base of a deep cleft separating the two major domains of the protein. This activity is greatly enhanced by polymerization of the actin (1). For those actins studied to date, hydrolysis occurs shortly after the incorporation of the actin monomer into the filament producing an ADP-P, species, which slowly loses its P to yield an ADP-monomer. Because ATP and ADP-P, F-actin are both more stable than ADP-F-actin, this P release is believed to play a major role in the treadmilling of actin filaments that occur within the cell.

Actin polymerization is a multiple-step process that includes a salt-induced G to F conformational change, rate-determining nucleation, and filament elongation until the monomer actin concentration reaches a limiting value called the critical concentration. Prior to incorporation of the actin monomer into the filament, it has been hypothesized that the actin proceeds through an F-monomer state (2). Evidence for such a species derives from experiments such as comparing protease digestion patterns of the actin in the absence and presence of polymerization-inducing salts at actin concentrations below the critical concentration of actin needed for formation of filaments. Using muscle actin, Shu et al. (3) determined that the F-monomer exhibits a low ATPase activity similar to that observed with G-monomer, suggesting that the enhanced ATPase activity associated with actin polymerization requires the conformation and inter-monomer contacts formed within the context of the actin filament.

We have previously created a mutant actin (GG-actin) in which we simultaneously converted Val266 and Leu267 to glycines (4, 5). These residues along with Leu 269 reside in the tip of a loop between actin subdomains 3 and 4. Holmes and co-workers (6) proposed that one major interstrand force stabilizing the actin filament was a “plug-pocket” interaction in which the tip of this loop interacts with a hydrophobic surface formed by the interface of two monomers on the opposing strand. In agreement with the predictions of this model, we have demonstrated that, as assessed by a change in light scattering, purified GG-actin alone fails to polymerize under normal polymerization conditions. The actin binding proteins tropomyosin (7) and fimbrin (8) can rescue GG-actin polymerization. However, this rescue is temperature-sensitive. In the presence of tropomyosin or fimbrin, polymerization of GG-actin occurs only at a temperature higher than 15 °C. Furthermore, when the temperature is lowered after polymerization reaches the steady state, depolymerization of actin filaments occurs.

The polymerization defect exhibited by GG-actin and the cold-sensitive rescue of GG-actin polymerization are consistent with the Holmes model. If the model is correct, removal of two hydrophobic residues at the tip of the plug should greatly decrease the proposed hydrophobic cross-strand interaction, leading to polymer instability of the mutant actin. Tropomyosin or fimbrin binding may strengthen intermonomer contacts along one strand partially compensating for the loss of cross-strand stabilization to the point that polymerization at 25 °C is restored. However, hydrophobic interactions are destabilized by colder temperatures (9). Thus, at low temperatures, the residual cross-strand interaction of the mutant actin is reduced to such an extent that the extra stabilization can no longer compensate, and filament formation cannot occur.

Although GG-actin has been used as a model system for examining the filament-stabilizing abilities of actin-binding proteins (7, 8), the specific mechanism responsible for the polymerization defect associated with GG-actin is unclear. For example, it is not known whether GG-actin remains in its G form under normal polymerization conditions, is converted to an F-actin monomer, or actually forms transient unstable F-actin oligomers. To understand the mechanisms by which this rescue of GG-actin polymerization occurs, it is necessary to first understand the nature of the polymerization defect. To explore...
these alternatives, we have assessed in this paper the effect of F buffer on the ATPase activity exhibited by GG-actin and the dependence of this activity on actin concentration and temperature. We have also determined the effect of temperature on the ATPase activity of WT yeast actin under polymerizing conditions.

MATERIALS AND METHODS

Actin Purification and Polymerization—Purification of wild-type and GG-actins from Saccharomyces cerevisiae was done by a combination of DNase I affinity chromatography and DEAE-cellulose chromatography as previously described (10). Affinity columns were made with DNase I purchased from Worthington and Affi-Gel 10 obtained from Bio-Rad Laboratories. Purified Ca\(^{2+}\)/G-actin was stored at 4 °C in Ca\(^{2+}\)/G buffer (10 mM Tris-HCl, pH 7.5, 0.2 mM CaCl\(_2\), 0.2 mM ATP, and 0.5 mM DTT) and used within 4 days.

Actin polymerization was induced by the addition of 2 mM MgCl\(_2\) and 50 mM KCl to a G-actin solution in G buffer (F buffer). Reactions of a total volume of 120 μl were followed in the thermostatted cuvette chamber of a fluorescence spectrometer. The increase in light-scattering caused by polymerization was monitored with the excitation and emission wavelengths set at 360 nm. For studies with the Mg\(^{2+}\) ion, actin was purified in the presence of EGTA in the presence of 0.1 mM MnCl\(_2\) at 4 °C for 10 min using a modification of a previously published procedure (11). Mg\(^{2+}\)/G-actin was used immediately after the conversion from Ca\(^{2+}\)/G-actin.

Enzchek Phosphate Assay—Release of inorganic phosphate from actin was quantitated by the Enzchek phosphate assay. This assay employs a kit obtained from Molecular Probes and is based on the P\(_i\)-dependent liberation of 2-amino-6-mercapto-7-methylpurine from methylthio-guanine, a nucleoside derivative of this base, by a purine nucleoside phosphorylase. The product base has a characteristic UV spectrum, not evident when part of a nucleoside, which can be utilized to monitor its phosphate-dependent rate of release by the enzyme. Each reaction mixture contained actin at the desired concentration as specified by the manufacturer, 2 units/ml purine nucleoside phosphorylase, and 200 μM methyl-thioguanine riboside. P\(_i\) release from actin in F buffer (10 mM Tris-HCl, pH 7.5, 0.2 mM ATP, 0.5 mM DTT, 2 mM MgCl\(_2\), and 50 mM KCl) or in Mg\(^{2+}\)-G buffer (10 mM Tris-HCl, pH 7.5, 0.2 mM ATP, 0.5 mM DTT, 2 mM MgCl\(_2\), and 50 mM KCl) or in Mg\(^{2+}\)-G buffer (10 mM Tris-HCl, pH 7.5, 0.2 mM ATP, 0.5 mM DTT, 2 mM MgCl\(_2\), and 50 mM KCl) was monitored continuously by the increase in absorbance at 380 nm. Phosphate standard curves were generated for each assay.

Tropomyosin Purification—Bovine cardiac tropomyosin was purified from cardiac actin powder according to the procedure of Butters et al. (12).

PDM Cross-linking—G-actin in G buffer was centrifuged through a micro Bio-Spin chromatography column (Bio-Rad) pre-equilibrated with DTT-free G buffer to remove remaining DTT, and the eluted G-actin was diluted to 20 μM with DTT-free G buffer. 20 μM of 20 μM actin was then incubated at 25 °C for 10 min with or without the addition of 2 mM MgCl\(_2\) and 50 mM KCl. A stock solution of 5 mM N\(_2\),N\(_2\)-1,4-phenylenediaminamide (PDM) in dimethylformamide was freshly diluted 10 times with DTT-free G buffer, and 1 μl of PDM was added into the 20-μl actin solution followed by an additional 8-min incubation. The final ratio of PDM to actin was about 1:1. The cross-linking was quenched by the addition of 20 μl of 2% SDS sample buffer containing 20% β-mercaptoethanol. The presence of cross-linked actin oligomers was analyzed by 10% SDS-PAGE.

RESULTS

P\(_i\), Release by GG-actin in F Buffer Is Significantly Faster Than in G Buffer—We first determined whether increasing the ionic strength to that normally used to induce actin polymerization causes by polymerization was monitored with the excitation and emission wavelengths set at 360 nm. For studies with the Mg\(^{2+}\) ion, actin was purified in the presence of EGTA in the presence of 0.1 mM MnCl\(_2\) at 4 °C for 10 min using a modification of a previously published procedure (11). Mg\(^{2+}\)/G-actin was used immediately after the conversion from Ca\(^{2+}\)/G-actin.

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RESULTS

P\(_i\), Release by GG-actin in F Buffer Is Significantly Faster Than in G Buffer—We first determined whether increasing the ionic strength to that normally used to induce actin polymerization enhances the ATPase activity of GG-actin by comparing P\(_i\) release from GG-actin under polymerization (F buffer) and monomeric (G buffer) conditions. Fig. 1 shows the time-dependent release of P\(_i\) from GG-actin with or without the addition of 2 mM MgCl\(_2\) and 50 mM KCl. Monomeric GG-actin exhibited a P\(_i\) release curve similar to that of WT actin. Addition of 2 mM MgCl\(_2\) and 50 mM KCl, which induces polymerization of WT-actin, did not do so with GG-actin as judged by a lack of change in light scattering and the absence of filaments in samples examined by electron microscopy (5). Interestingly, however, the rate of P\(_i\) release from GG-actin in F buffer increased 4-fold compared with that in G buffer.

P\(_i\), Release by GG-actin Is Greatly Increased Above a Certain Actin Concentration—To explore the mechanism behind this enhanced GG-actin ATPase activity in the presence of F buffer, we first measured the continuous release of P\(_i\) from WT-actin to which F salts had been added. Our results demonstrate (see Fig. 6) that P\(_i\) release from WT-actin closely mirrors its polymerization curve reaching a plateau when F-actin formation reaches equilibrium. We next measured P\(_i\) release by Mg\(^{2+}\)-GG-actin at different concentrations under polymerization conditions (Fig. 2A). At low concentrations, a continuous monophosphoric P\(_i\) release occurred. However, at higher actin concentrations, P\(_i\) release by GG-actin appeared to be biphasic: a lag phase and a linear fast release phase with no plateau. We observed the same behavior with Ca\(^{2+}\)/GG-actin, showing that this behavior, at least qualitatively, was independent of the nature of the tightly bound divalent cation (Fig. 2B).

This type of concentration-dependent P\(_i\) release by GG-actin resembles a critical concentration curve determination for actin polymerization, suggesting that GG-actin might form unstable oligomers leading to abortive polymerization. For both cations, we thus plotted the rate of P\(_i\) release by determining the slopes of the linear second phase of each curve as a function of actin concentration (Fig. 3). This plot exhibited two linear phases as a function of actin concentration, and the intersection of the two lines yielded an apparent critical concentration for enhancement of ATPase activity of ~8 μM for Mg\(^{2+}\)-GG-actin. A duplicate experiment with a second actin preparation yielded an apparent critical characterization of 7 μM (data not shown). Both determinations using Ca\(^{2+}\)-GG-actin yielded a critical concentration value of 11 μM. This distinction in apparent critical concentration for enhancement of P\(_i\) release is consistent with the divalent cation-dependent difference in critical concentration for polymerization determined earlier for muscle actin (13), although the difference is smaller than that reported in this previous study. Our results therefore indicate that the enhanced ATPase activity we observed for GG-actin in F buffer results not merely from conversion of a G- to an F-monomer but derives from the formation of unstable oligomers of F-actin.

It has previously been shown (14) that, for a polymerization process like that of actin, a plot of ln (lag phase) versus ln (actin) will produce a line with a slope equal to one-half of the number of monomers in the nucleus. Using the data in Fig. 2...
for Mg\textsuperscript{2+}-actin, we calculated the lag phase by determining the point at which the second linear part of the curve intersected the first section of the plot for the 10, 12, and 16 μM curves. The plot of ln (lag phase) versus ln [actin] shown in Fig. 4 is a straight line with a slope of 2.1. This experiment was repeated with a second preparation of actin, and a slope of 1.85 was obtained (data not shown). This result, consistent with the data of Tobacman and Korn for muscle actin (14), indicates that the increase in ATPase activity following addition of salt depends on the formation of a nucleus with three or four monomers, further demonstrating that it results from abortive F-actin formation.

**Temperature Dependence of P\textsubscript{i} Release by Polymerizing WT Actin**—It is possible that the temperature sensitivity of P\textsubscript{i} release by GG-actin reflects an inherent sensitivity of the ATPase reaction per se rather than temperature-sensitive actin oligomerization. We thus assessed both the extent of polymerization and the P\textsubscript{i} release by WT yeast actin as a function of temperature following induction of polymerization by the addition of Mg\textsuperscript{2+}-GG-actin; *, 8 μM GG-actin; ○, 10 μM GG-actin; +, 4 μM GG-actin. This experiment was repeated with two different preparations of actin with essentially similar results.

**Estimation of nucleus size for Mg\textsuperscript{2+}-GG-actin oligomerization.** The lag times for the fast release phase of 10, 12, and 16 μM Mg\textsuperscript{2+}-GG-actin were determined by the intersection of the two phases. The ln (lag time) was plotted versus ln [actin], yielding a straight line with a slope of 2.1 and a correlation coefficient of 0.99. A repetition of this experiment with a second preparation of actin yielded a slope of 1.85 with a correlation coefficient of 1.
diation of salts. Fig. 6 shows that decreasing the temperature results in a concomitant decrease in both the rate of polymerization and Pi release. ATPase activity is present at all temperatures, and the extent of Pi release from WT actin is governed solely by the extent of actin polymerization. These results suggest that the decrease in ATPase activity observed reflects a decrease in the rate of polymerization and not an inherent temperature-sensitive decrease in the ATPase activity per se. At 15 °C, where P_i release from GG-actin had disappeared, WT actin exhibited significant P_i release with its rate dependent solely on the rate of polymerization of the actin. Therefore, the lack of P_i release, and thus ATPase activity, seen with GG-actin at 15 °C most likely results from the inability of this actin to nucleate and form transient F-actin oligomers.

Although it has been demonstrated that muscle actin slowly releases its bound P_i after ATP hydrolysis (15), it is not known whether or not yeast actin has the same behavior. To address this question, we normalized the data from light scattering and P_i release at 25 °C and plotted them together (Fig. 6). The slope for the fast release phase at each temperature was replotted as a function of temperature. C, the slope for the slow release phase at each temperature was replotted as a function of temperature. D, the release rate at each temperature relative to that at 25 °C for each phase was plotted. □, slow phase; ○, fast phase. This experiment was repeated with two different actin preparations with same results.

though Shu et al. (3) reported that the F-monomer did not exhibit enhanced ATPase activity over the G-monomer, their study was performed for very short times following addition of salt to actin solutions above the critical concentration, which might have led to inaccuracies in their measurements. Second, in a number of instances dealing with polymerization rates and nucleotide exchange, yeast and muscle actins have been shown to behave quantitatively quite differently (4, 16). Fig. 3 shows that oligomerization of GG-actin only occurs when its concentration is above the critical concentration. This suggests that we could possibly gain a population representing F-monomeric actin when we induce the conformational switch by F salts with GG-actin at concentrations below the critical concentration. Thus, we determined whether, below the critical concentration, GG-actin in F buffer would display an enhanced ATPase activity over that seen in G buffer. We incubated 5 μM Mg\(^{2+}\)-GG-actin at 25 °C with or without the addition of salts and determined the rate of P_i release by the Enzcheck phosphate assay. No difference could be seen between these two salt conditions in agreement with the results of Shu et al.

Probing GG-actin Oligomer Formation by Cross-linking—N,N'-1,4-Phenylenedimaleimide (PDM) has been used to probe the oligomeric state of actin polymerization. Millonig and Aebl (17) have demonstrated the time-dependent appearance of two cross-linked species during polymerization, a lower dimer (LD) with an apparent molecular mass of 86 kDa and an upper dimer (UD) with an apparent molecular mass of 115 kDa by SDS-PAGE. The LD, a Cys374-Cys374 cross-linked species (18), appears immediately after salt induction and is not compatible with the structure of the actin filament. The UD forms between Lys191 on one subunit and Cys374 on another subunit along the helical filament and reflects the structure of a dimer in the actin filament. The LD is consumed during polymerization probably via two pathways (19). In one path, the LD is in equilibrium with a G-actin pool and indirectly involved in polymerization. In a second pathway, during polymerization LD directly incorporates into actin filaments to form LD-decorated F-actin. The unincorporated actin subunits are then released...
during filament maturation probably due to an F-conformational change resulting in smooth-looking filaments.

We monitored GG-actin oligomerization by PDM cross-linking. 20 μM Ca²⁺-GG-actin induced by the addition of 2 mM MgCl₂ and 50 mM KCl at different temperatures is shown using light scattering as an assay. B, P_i release under the same conditions, as monitored by the Enzcheck assay, is shown. □, 25 °C; ×, 20 °C; ○, 15 °C; ○, 10 °C; +, 7 °C. C, the data from light scattering and P_i release at 25 °C were normalized and plotted together. □, light scattering; ○, P_i release. This experiment has been repeated three times with different preparations of actin with essentially the same results.

**DISCUSSION**

We previously demonstrated that, in agreement with Holmes’ model of the actin filament, eliminating hydrophobicity in a subdomain 3/4 loop in yeast actin (GG) resulted in a nonpolymerizing protein (5). However, we showed that polymerizability could be restored by incubation with phalloidin (5), Sac6p (8), or tropomyosin (7). Whether the polymerization defect lay in the nucleation or elongation phase could not be determined. The demonstration that BeFx, a phosphate analogue, also restored polymerization (5) suggested that the protein could still hydrolyze ATP. However, BeFx-dependent rescue of polymerization could have resulted from stabilization of labile oligomers following nucleation or by generation of ADP-BeFx actin, which then polymerized due to an altered conformation.

We have addressed these questions by employing a continuous phosphate release assay to assess nucleotide hydrolysis by GG-actin. This assay provides a combined estimate of the hydrolysis of ATP and the subsequent release of the Pi from the actin, although it does not allow us to examine the ATP hydrolysis step per se. However, the virtual coincidence of the polymerization and P_i-release curves for yeast actin makes it very unlikely that P_i release is rate-limiting in comparison to ATP hydrolysis, thereby validating this approach.

Although GG-actin will not form stable filaments, our results...
demonstrate a substantial F buffer-dependent increase in GG-actin ATPase activity for both the Ca\(^{2+}\) and Mg\(^{2+}\) forms of the actin. In contrast with the case of WT actin, however, the ATPase activity of GG-actin is continuous. Theoretically, the continuous nature of this activity could result from an F-monomer ATPase, since previous work had presented evidence for the generation of such a species in solution (2). Alternatively, continuous activity could result from the formation and cycling of small oligomers, undetectable by light scattering. Our results demonstrate that the second alternative is the correct one. In the case of WT actin polymerization, continuous low level linear ATPase activity due to treadmilling occurs at the steady state. In response to the introduction of F salts into solution, GG-actin exhibits a biphasic ATPase activity, an initial low linear ATPase activity followed by a faster but still linear phase. The low ATPase activity is very likely due to a process involving nucleation. The second faster phase could not result from a typical net elongation phenomenon, because this would require the attainment of a steady-state level of filament formation that does not occur. Instead, the continuous activity probably reflects a steady state of exchange of monomers from unstable oligomers driven by the hydrolysis of ATP. In essence, the system is one in which, once an apparent critical concentration for GG-actin is reached, nuclei or short oligomers provide a surface catalyst for the hydrolysis of ATP by cycling monomers. Because this is a steady-state situation governed by the critical concentration of the system, as the actin concentration increases, the filament ends, but not the free monomers, increase concomitantly leading to the actin-dependent linear increase in ATPase activity we observe.

The apparent critical concentration of GG-actin, about 11 \(\mu M\) for \(\text{Ca}^{2+}\)-actin and 7 or 8 \(\mu M\) for \(\text{Mg}^{2+}\)-actin is \(~50–60\) times higher than it is for WT actin. Wen (7) had previously determined a critical concentration of 5.5 \(\mu M\) for the tropomyosin-dependent rescue of \(\text{Mg}^{2+}\)-GG-actin polymerization by light scattering. The small difference (0.086 mg/ml) between the critical concentration of intrinsic oligomerization and tropomyosin-dependent polymerization may be due to different preparations of actin or small variations in carrying out the experiments. It is also possible that tropomyosin slightly decreases the critical concentration of GG-actin polymerization. However, the measurement of an intrinsic critical concentration for GG-actin oligomerization allows for the first time an estimate of the importance for filament stabilization of the hydrophobicity normally associated with Val\(^{266}\) and Leu\(^{267}\), which was eliminated in this mutant. Furthermore, this system provides us with a way of actually assessing nucleation and oligomerization of actin under conditions that do not permit formation of stable filaments.

Demonstration of an oligomerization-dependent ATPase coupled with our previous finding that BeFx allows formation of stable GG-actin filaments allows one to understand the underlying basis for the instability of GG-actin to form stable filaments alone. There is clearly an effect on the critical concentration of the ATP-actin. In other words, in terms of the Holmes model, in the ATP monomer, the residual hydrophobicity of the plug is oriented correctly enough to provide sufficient cross-strand stabilization for initiation of actin filament formation. However, ATP hydrolysis and subsequent release of the phosphate alters the conformation of either the plug or other monomer contacts enough so that this residual plug hydrophobicity is no longer sufficient for maintenance of the filament structure. In other words, the critical concentration of the ADP-actin monomer is so high that, under the conditions of the experiment, filaments cannot form.

Oligomerization of GG-actin was directly demonstrated by PDM cross-linking. Under conditions where steady ATPase activity is observed, GG-actin forms a significant amount of LD along with much less UD. Because the UD is the precursor for nucleus and filament formation, presumably it is the conformation responsible for the F-like ATPase activity. Taking into account the differences between the critical concentrations of WT- and GG-actin, there can be no more than half as much GG F-actin species as WT species at any point. The fact that the ratio for UD between these two actins is much greater than 2:1 can be attributed to the continuous cycling of the GG F species and a resulting reduction in the frequency with which they can react with the cross-linker and perhaps to an inherently less reactive conformation assumed by the GG-actin per se. This result substantiates our previous hypothesis that tropomyosin-dependent rescue of GG-actin polymerization depends initially on the capture of pre-formed actin oligomers and not on tropomyosin-dependent nucleation of filament formation.

Our temperature studies not only provide additional information concerning the effect of the GG mutation on actin function, but they also provide new insight into the ATPase of WT actin as well. The extent of polymerization of WT actin is minimally affected by cold temperatures, although, as the temperature drops, the rate of filament formation slows. Our demonstration that the rate and extent of P, release during this process closely parallels the rate of formation of F-actin indicates, for the first time, that the F-actin ATPase activity per se is not cold-sensitive. This situation is contrary to what is found with many enzyme-substrate systems in which cold temperatures do not inhibit substrate binding but do inhibit catalysis.

With 12 \(\mu M\) \(\text{Mg}^{2+}\)-GG-actin, F-actin-dependent ATPase activity was not observed unless the temperature was \(>15\) °C. This cold-sensitive behavior of GG-actin alone allows us for the first time to compare the effects of this double mutation quantitatively with the less severe polymerization defects observed with other alterations in the hydrophobic plug. The L267D mutant, in which a negative charge was placed between the two remaining hydrophobic groups required \(>6\) °C for polymerization, whereas little if any effect was observed when either Val\(^{266}\) or Leu\(^{267}\) was altered singly to G or when Val\(^{266}\) was substituted with D (21).

P, release is the slow step in the nucleotide cycle that occurs during the polymerization of muscle actin (1), and the lag between ATP hydrolysis and P, release has been proposed to be an important factor in the rate of actin filament turnover (22). In this light, our observation that such a lag did not exist with yeast actin was very surprising and suggests the possibility of a comparatively greater degree of instability of yeast actin filaments compared with those of higher eukaryotic actsins. We previously observed that nucleotide exchange in yeast actin is faster than in higher eukaryotic actsins (4, 23). Furthermore, when we compared the rate of release of P, by monomeric yeast and muscle actsins (data not shown), the rate constant for the reaction in yeast G-actin (\(2.8 \times 10^{-4}\) s\(^{-1}\)) is faster than that exhibited by muscle actin (\(9 \times 10^{-5}\) s\(^{-1}\)). These findings reinforce the suggestion of an inherently less stable yeast actin filament in comparison with that of higher eukaryotic actsins.

Systematically studying the ATPase activity that accompanies actin polymerization is inherently difficult because of the self-limiting nature of the reaction. Its rate slows drastically as polymerization reaches the steady state requiring that large amounts of actin be employed if reasonable signals are to be obtained. This problem can be circumvented somewhat by employing continuous sonication to make the ATPase reaction continuous. However, the awkwardness of this approach coupled with artifacts arising from the heat generated by the sonic probe makes this approach less than desirable. The continuous ATPase activity exhibited by GG-actin in F buffer potentially
provides us with a means for experimentally addressing the mechanism of the actin-dependent hydrolysis of ATP under polymerizing conditions.

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