Polymerization and *in Vitro* Motility Properties of Yeast Actin: A Comparison with Rabbit Skeletal α-Actin†

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ABSTRACT: Actin purified from the yeast (*Saccharomyces cerevisae)* was polymerized faster than rabbit skeletal α-actin by $MgCl_2$. The two actins polymerized at similar rates in the presence of $CaCl_2$. Yeast actin, up to 25 μM, was not polymerized by KCl (100−300 mM); the monovalent salt also inhibited the $MgCl_2$-induced polymerization of actin. The local structure of the subdomain-2 region in yeast actin filaments was probed by subtilisin and trypsin digestions. Loop 38−52 appeared more flexible and accessible to subtilisin in yeast than in rabbit actin. In contrast, tryptic digestions at Lys-61 and -68 occurred at the same rate for yeast and α-actin filaments. Modification of yeast actin by a sulfhydryl reagent CPM [7-(diethylamino)-3-(4′-maleimidophenyl)-4-methylcoumain] was specific to the Cys-374 residue; no labeling of a yeast actin mutant containing an alanine substitution for cysteine 374 was observed. The rates of Cys-374 labeling by CPM were similar for yeast and muscle actin, suggesting a similar environment for the C terminus in both polymers. In the *in vitro* motility assays, yeast actin required higher concentrations of heavy meromyosin (HMM) for its sliding than did the rabbit actin. At saturating concentrations of HMM, the sliding velocities of both actins were the same (3.0 μm/s). Relative forces generated by HMM with yeast and muscle actin were assessed by monitoring their *in vitro* motility in the presence of NEM−HMM load. The sliding of yeast actin was stopped at a level of external load (molar ratio NEM−HMM/HMM = 0.25) lower than that of muscle actin (NEM−HMM/HMM = 0.43), suggesting lower force production with yeast actin. These results are discussed in terms of the myosin cross-bridge cycle and actomyosin interactions.

In recent years, budding yeast (*Saccharomyces cerevisae*) has emerged as a powerful system for studying the structure and function of actin and the actin cytoskeleton (Amberg et al., 1995). The main reason for this development is the relatively easy creation and isolation of mutant actins that can then be analyzed by cell biological, biochemical, and genetic techniques. To date, this approach has been used for examining the interaction of actin with proteins such as myosin, fimbrin, tropomyosin, profilin, and gelsolin as well as with actin itself (Miller et al., 1995, 1996a; Amberg et al., 1995; Nefsky & Bretscher, 1992; Chen et al., 1993, 1995; Buzzan & Frieden, 1996). The actin sequence and its biochemical properties appear to be well conserved, indicating that principles elucidated from studies with the yeast system should have general applications and validity.

Despite 87% sequence identity, some functional differences between wild type yeast actin and rabbit α-actin have been observed (Greer & Schekman, 1982; Kron et al., 1992; Buzan & Frieden, 1996). In a recent study (Buzan & Frieden, 1996), but not in an earlier work (Nefsky & Bretscher, 1992), the polymerization of these two actins by $MgCl_2$ was found to be different. The wild type yeast actin polymerized at a faster rate than muscle actin, apparently because of differences in the nucleation step of the reaction. Also, to account for the kinetics of yeast actin polymerization, Buzan and Frieden (1996) assumed a fragmentation of actin filaments. This, in turn, implies that some intermolecular contacts along the filament axis may be weaker for yeast than for muscle actin. A similar conclusion has been reached on the basis of the electron microscopy of yeast actin filaments (Orlova et al., 1996) and is supported also by the much greater fragmentation of yeast than of muscle actin filaments in all of our *in vitro* motility assays.

Of particular interest are the functional interactions of yeast actin with myosin. The binding of myosin to wild type yeast actin was documented by Greer and Schekman (1982). In the absence of nucleotides, such rigor myosin binding was much weaker to yeast actin than to muscle actin (Kron et al., 1992). Subsequent work showed that the rigor binding of myosin subfragment-1 (S1)[1] to yeast actin is about 10-fold weaker than to muscle actin (Miller et al., 1995), but the weak state binding, in the presence of MgATP, is approximately the same for the two actins (Cook et al., 1993). Despite similar weak binding affinities for myosin, the yeast actin activates myosin ATPase activity much less than muscle actin (Kron et al., 1992) due to a several-fold lower V_{max} value for that reaction. The *in vitro* motilities of yeast and muscle actins over myosin were reported to be the same (Cook et al., 1993). However, this result differed from the previous conclusions of Kron et al. (1992), who measured a 50% slower sliding of yeast actin than muscle actin over an

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[1] Abbreviations: DNaseI, deoxyribonuclease I; F-actin, filamentous (polymerized) actin; G-actin, monomeric actin; HMM, heavy meromyosin; S1, myosin subfragment-1; NEM, *N*-ethylmaleimide; CPM, 7-(diethylamino)-3-(4′-maleimidophenyl)-4-methylcoumarin.

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S1-coated surface. The present use of yeast actin as the system of choice for mutational analysis of actomyosin interactions and motility requires the clarification of such differences and a more detailed understanding of the *in vitro* motility of yeast actin.

Accordingly, the first goal of this study was to compare the polymerization reactions of yeast and muscle actins and to test some of the possible reasons for the different stability of their filaments. Our second goal was to compare the mechanical performance of yeast and muscle actins in the *in vitro* motility assays.

MATERIALS AND METHODS

Reagents. Distilled and Millipore-filtered water and analytical grade reagents were used in all experiments. ATP, PMSF, DTT, phalloidin, *N*-ethylmaleimide, rhodamine phalloidin, and β-mercaptoethanol were purchased from Sigma Chemical Co. (St. Louis, MO). DNaseI was purchased from Boerhinger Mannheim (Indianapolis, IN). *N*-(1-Pyrenyl)-iodoacetamide and 7-(diethylamino)-3-(4′-maleimidophenyl)-4-methylcoumarin (CPM) were obtained from Molecular Probes (Eugene, OR).

Preparation of Proteins. Yeast actin was isolated using DNaseI affinity chromatography as previously described (Cook et al., 1993). Rabbit actin and myosin were prepared from rabbit skeletal muscle according to the methods of Spudich and Watt (1971) and Godfrey and Harrington (1970), respectively. Myosin subfragment-1 was prepared according to Weeds and Pope (1977).

Polymerization of Actins. The polymerization of G-actin by $MgCl_2$, $CaCl_2$, and KCl in G-actin buffer [0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM β-mercaptoethanol, and 5.0 mM Tris-HCl (pH 7.6)] was observed by measuring the light scattering of actin solutions at 325 nm in a Spex Flourolog spectrophotometer (Spex Industries Inc., Edison, NJ). The final light scattering values for the polymerized actin were measured after an overnight incubation of samples at 4 °C. To exchange Ca^{2+} with Mg^{2+} in G-actin, Ca^{2+}−G-actin was incubated with 400 μM EGTA and 100 μM $MgCl_2$ for 10 min. All light scattering and fluorescence results are presented in arbitrary units (au).

Pyrene and CPM Modification of Yeast and α-Actins. Yeast and rabbit α-actin were labeled at Cys-374 with N-(1-pyrenyl)iodacetamide following the method of Cooper et al. (1983). CPM [7-(diethylamino)-3-(4′-maleimidophenyl)-4-methylcoumarin] modification of actin was carried out as described by Muhlrad et al. (1994). The progress of actin labeling was monitored via CPM fluorescence in a Spex fluorolog, with the excitation and emission wavelengths set at 387 and 470 nm, respectively.

Proteolytic Digestion of F-Actin. Digestions of 1.0 mg/mL rabbit and yeast F-actin were performed in 5.0 mM Tris-HCl, 2.0 mM $MgCl_2$ (pH 7.7), 0.5 mM β-mercaptoethanol, 0.2 mM ATP, and 0.2 mM $CaCl_2$. The digestions (at 23 °C) were started with the addition of subtilisin (0.04 mg/mL) at a weight ratio of 25/1 (actin/protease). At given time intervals, aliquots were removed from the mixture and the reactions were stopped by addition of 10 mM PMSF. Digestions of F-actin by trypsin were done at a weight ratio of 5/1 (actin/trypsin) and were performed similarly, except for the addition of soybean trypsin inhibitor (3 × the trypsin concentration, w/w) to stop the reactions. The denatured samples were run on SDS−PAGE, and the Coomassie blue-stained bands of uncleaved actin were quantified by laser densitometry. Cleavage rates were obtained from semilogarithmic plots of the undigested, intact actin versus the time of digestion.

In Vitro Actin Motility Assays. The motility assays were performed as previously described (Miller et al., 1996a). The temperature was maintained at 25 °C for all assays. HMM was prepared as described by Kron et al. (1991). In order to remove ATP-insensitive heads, HMM was centrifuged with 0.15 mg/mL phalloidin-stabilized actin in a solution containing 0.1 M KCl, 4 mM $MgCl_2$, and 3 mM MgATP for 20 min in a Beckman airfuge. Rhodamine phalloidin-labeled actin filaments were added to the HMM-coated cover slips at 10 nM, and after 1 min, the unbound filaments were washed away with the assay buffer [25 mM KCl, 1 mM EGTA, 4 mM $MgCl_2$, 10 mM dithiothreitol, and 10 mM imidazole (pH 7.4)]. Movement was initiated with the assay buffer containing 1 mM ATP and an oxygen-scavenging system. Quantification of the sliding velocities was done with an Expertvision system (Motion Analysis, Santa Rosa, CA). The velocities of individual filaments with standard deviations of less than $^1/_3$ of the average velocity were used for statistical analysis (Homsher et al., 1992) and were considered to move smoothly in the assay system.

NEM−HMM was prepared as described previously (Warrick et al., 1993). The *in vitro* motility assays were performed as above with the following modifications. HMM and NEM−HMM at appropriate weight ratios were adsorbed to the assay surface for 2 min and washed off with 6 volumes of assay buffer to prevent contamination with NEM−HMM. Nonmoving filaments were determined by tracking the centroid of each filament image with the Expertvision system and charting its movement for 20 s.

RESULTS

Polymerization of Yeast Actin. The polymerization of G-actin by $MgCl_2$, $CaCl_2$, and KCl was monitored by light scattering measurements. In agreement with a recent report (Buzan & Frieden, 1996), 3.0 mM $MgCl_2$ polymerized the Ca^{2+}−G-actin from wild type yeast faster than that from the rabbit skeletal muscle (Figure 1, solid traces). This difference in the polymerization rates of yeast and muscle actins decreased when the tightly bound Ca^{2+} in G-actin was replaced with Mg^{2+} prior to the polymerization (Figure 1, dotted traces). The same final plateau levels of light scattering were reached for both yeast and muscle actins. The rates and the extent of Ca^{2+}−G-actin polymerization by $CaCl_2$ were virtually the same for the two actins (data not shown).

In contrast to Mg^{2+}- and Ca^{2+}-induced polymerizations of G-actin, the reaction stimulated by monovalent salt was qualitatively different for yeast and muscle actins. As shown in Figure 2a, no polymerization of yeast actin (up to 25 μM) was detected after addition of 0.1 M KCl to Ca^{2+}−G-actin, even after 18 h of incubation at 4 °C (Figure 2c). Pelleting experiments confirmed this observation. After ultracentrifugation (in a Beckman airfuge) of an actin sample (25 μM) incubated overnight with 0.1 M KCl, all the yeast actin was recovered in the supernatant while most of the muscle actin (∼90%) was found in the pellet.

Figure 2b shows that KCl also inhibits the Mg^{2+}-induced polymerization of yeast actin (3.0 μM). The presence of

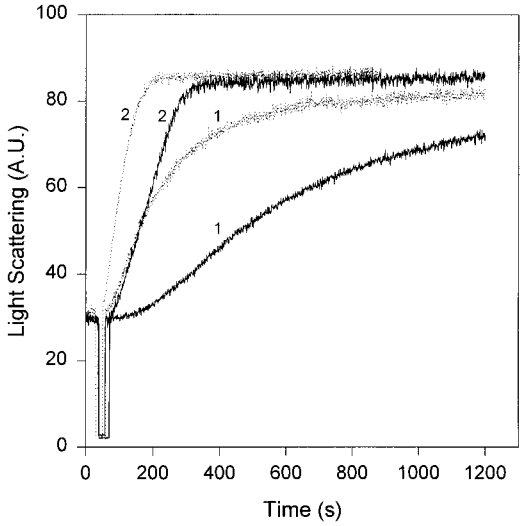


FIGURE 1: Light scattering record of $MgCl_2$-induced polymerization of actin. Rabbit skeletal α-actin and yeast actin were polymerized by the addition of 3.0 mM $MgCl_2$ to 5.0 μM actin in G-actin buffer. The polymerization was followed for Ca^{2+}−G-actins supplied with $MgCl_2$ (solid traces) and the Mg^{2+}−G-actins, after exchange of Ca^{2+} with Mg^{2+} (dotted traces). Traces 1 and 2 correspond to α-actin and yeast actin, respectively.

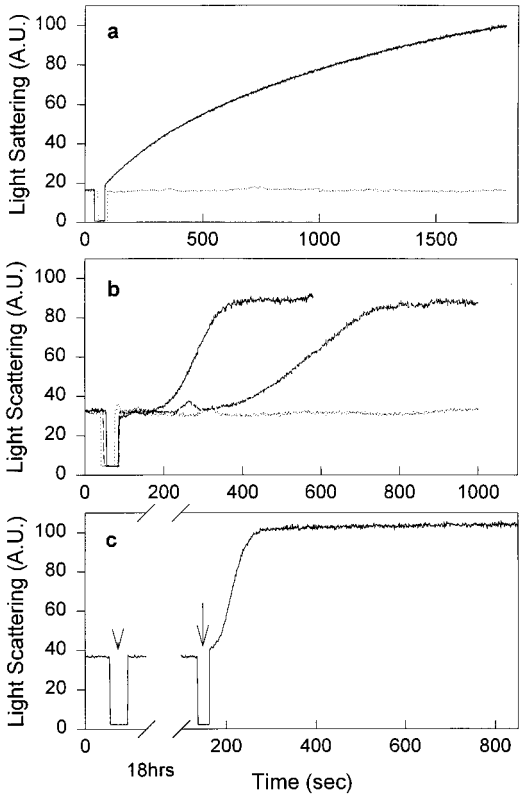


FIGURE 2: Light scattering measurements of actin polymerization in the presence of KCl. (a) KCl (100 mM) was added to α-actin (25 μM) (solid trace) and yeast actin (dotted trace) in G-actin buffer. (b) Polymerization of 3.0 μM yeast actin by 3.0 mM $MgCl_2$ in G-actin buffer in the absence of KCl (solid trace) and in the presence of 100 mM (middle trace) and 300 mM KCl (dotted trace). (c) Light scattering from yeast actin solution (10 μM) in G-actin buffer before and after addition of 100 mM KCl, after an 18 h incubation with 100 mM KCl at 4 °C, and after the addition of 3.0 mM $MgCl_2$. The arrowhead shows the addition of KCl, and the arrow indicates the addition of $MgCl_2$.

0.1 M KCl in the polymerization mixture (middle curve in Figure 2b) increased the time lag for the onset of polymerization, revealing the effect of monovalent salt on the nucleation step of the reaction. The addition of 0.3 M KCl inhibited even more the Mg^{2+}-induced polymerization of yeast actin (dotted curve in Figure 2b). The inhibition of yeast G-actin polymerization by KCl was not associated with any irreversible changes in actin. Addition of 3.0 mM $MgCl_2$ to yeast actin (10 μM) which was incubated overnight with 0.1 M KCl resulted in the polymerization of this actin (Figure 2c). The effect of KCl on yeast actin was not caused by one of the steps in its purification on the DNaseI column. Rabbit skeletal α-actin passed through the same column was polymerized by 0.1 M KCl as well as the control actin.

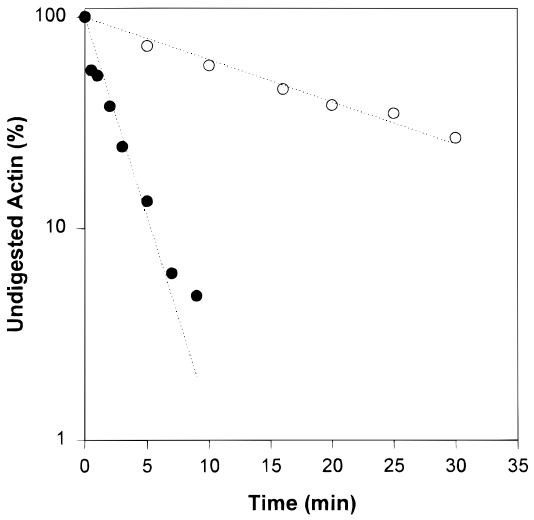


FIGURE 3: Semilogarithmic plot of subtilisin cleavage of F-actin versus time of digestion. Subtilisin (0.04 mg/mL) was added to 1.0 mg/mL muscle F-actin (○) and yeast F-actin (●) in G-actin buffer containing 2 mM $MgCl_2$. The progress of digestions (at 23 °C) with time was monitored by densitometric analysis of the uncleaved actin bands on SDS−PAGE. The first-order rate constants for the cleavage of muscle and yeast F-actin were 0.046 ± 0.005 and 0.44 ± 0.15 min^{-1}, respectively.

Proteolytic Cleavage of Subdomain-2. The polymerization experiments revealed that the main difference between the yeast and muscle actins was in the assembly of Ca^{2+}−G-actin by KCl (or NaCl). It has been observed before that the KCl-driven polymerization of muscle Ca^{2+}−G-actin was more sensitive to the integrity of loop 38−52 (the DNaseI loop) on actin than the Mg^{2+}- or Ca^{2+}-induced reactions (Schwyter et al., 1989; Khaitlina et al., 1993). The cleavage of muscle G-actin by subtilisin, between Met-47 and Gly-48, and by a bacterial protease ECP (from *Escherichia coli* A2 strain), between Gly-42 and Val-43, increased greatly the critical concentration for KCl polymerization of Ca^{2+}−G-actin and much less for the Mg^{2+}-induced reaction. This tentative correlation raised the possibility that intermolecular contacts of subdomain-2 are less favored in yeast than in muscle actin filaments. To test this hypothesis, the rates of proteolytic digestion of subdomain-2 by subtilisin and trypsin were compared for the yeast and muscle F-actins. As shown in Figure 3, the rate of subtilisin cleavage of yeast actin filaments (k = 0.44 ± 0.15 min^{-1}), as monitored by the disappearance of the intact, undigested actin bands on SDS−PAGE, was about 10-fold faster than that of muscle F-actin (0.046 ± 0.005 min^{-1}). Because of the identical sequence of loop 38−52 in both actins, this result suggests that the dynamic loop 38−52 is less frequently in contact with the

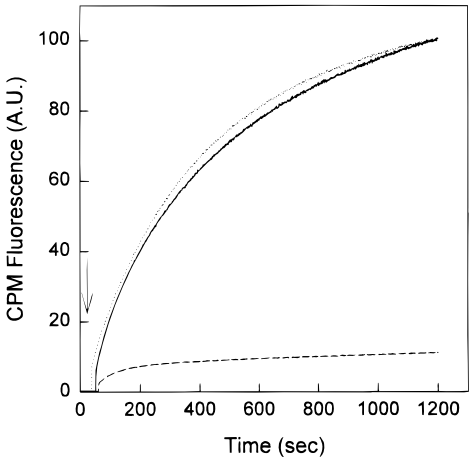


FIGURE 4: Fluorescence measurements of CPM modification of Cys-374 on actin. F-Actin (10 μM) in G-actin buffer free of β-mercaptoethanol and containing 2.0 mM $MgCl_2$ was mixed with 8.0 μM CPM at 22 °C. The modification of actin was monitored via the increase of CPM fluorescence (λ_{ex} = 387 nm, λ_{em} = 470 nm) for the reactions with muscle actin (solid curve), wild type yeast actin (dotted curve), and C374A yeast mutant actin (dashed curve).

adjacent actin and is more exposed to proteolytic attack in yeast than in muscle F-actin.

Similar tryptic digestions of Mg^{2+}-polymerized yeast and muscle actin filaments did not reveal significant differences in the susceptibility of these proteins to trypsin. Since trypsin attacks mainly at Lys-61 and Lys-68 in F-actin (Jacobson & Rosenbusch, 1976), it would appear that the differences in the environment of loop 38−52 in yeast and muscle F-actin do not extend to the Lys-61−Lys-68 region of subdomain-2.

Modification of the C Terminus of Actin. Another region on actin which appears to influence filament formation and stability is its C terminus (O'Donoghue et al., 1992; Mossakowska et al., 1993). The C terminus may be involved in intermolecular interactions of actin in the filament (Holmes et al., 1990; Lorenz et al., 1993; Owen & DeRosier, 1993; Tirion et al., 1995), and there is evidence for its intramolecular (Kuznetsova et al., 1996) and intermolecular (Kim & Reisler, 1996) coupling to the 38−52 loop. The environment of the C terminus on yeast actin was probed by monitoring the modification of its penultimate Cys-374 residue via fluorescence measurements. As shown by changes in fluorescence, the rates of Cys-374 labeling by CPM in yeast and in muscle F-actin were virtually identical (Figure 4).

To verify that in analogy to α-actin Cys-374 is the only cysteine residue modified by CPM in yeast actin (under these conditions), the labeling reaction was monitored also for a C374A yeast actin mutant in which Cys-374 was replaced with alanine (purified from a yeast strain constructed by P. A. Rubenstein). As observed before (P. A. Rubenstein, private communication), neither fluorescence measurements (Figure 4) nor examination of actin bands on SDS−PAGE under UV illumination (not shown) revealed any incorporation of CPM into the C374A yeast actin. Thus, the similar rates of yeast and muscle F-actin labeling by CPM (Figure 4) suggest a similar environment of Cys-374 in these polymers. However, small differences in the environment cannot be ruled out, considering the observation that the usual enhancement of pyrenyl actin fluorescence upon polymerization of Cys-374 pyrene-labeled muscle G-actin (15−20 times) is significantly smaller for pyrenyl yeast G-actin (8−10 times) (data not shown). The quenching of pyrenyl F-actin fluorescence by the bound S1 was similar (∼70%) for both actins.

Table 1: Effect of HMM Concentration on the Sliding of Yeast and α-Actin Filaments in the *in Vitro* Motility Assays[a]

	sliding velocity (μm/s)	
HMM (mg/mL)	rabbit α-actin	wild type actin
0.25	3.0	2.9
0.12	3.1	2.6
0.05	2.4	1.9
0.02	2.1	0
0.01	0	na

[a] The motilities of actin filaments were measured as described in Materials and Methods. At least 50 filaments were analyzed for each sample. The standard deviation of the mean values was between 0.1 and 0.3 μm/s. The concentrations of HMM are for solutions used for adsorption of HMM to cover slips. na, not available.

In Vitro Motility of Yeast Actin. In agreement with previous results of Cook et al. (1993), the sliding velocities of yeast and muscle actin filaments were the same under standard conditions of the *in vitro* motility assays, i.e., at low salt (I = 50 mM) and in the presence of a saturating density of HMM on the cover slip (Table 1). Under these conditions, the two features which distinguish yeast from muscle actin filaments are the much greater fragmentation of the former and the lower binding stability of rhodamine phalloidin with yeast actin (DeLaCruz & Pollard, 1996). However, as documented in the companion work on yeast actin mutants (Miller et al., 1996b), the standard measurements may be unable to resolve the differences between different actins. Indeed, when the *in vitro* motilities were measured over a range of HMM concentrations, the yeast actin filaments "underperformed" in comparison to α-actin filaments at low HMM concentrations (Table 1). Yeast actin filaments required higher HMM amounts than did muscle actin to support their motion and maintain comparable velocities of sliding. A simple interpretation of this result, in line with the analysis of mutant actin motilities in the companion study (Miller et al., 1996b), is that a smaller number of force-generating cross-bridges is formed with wild type yeast than with muscle actin. At lower densities of HMM on the surface, this leads to a greater inhibition of yeast than muscle actin motion in the motility assays.

Qualitative but readily attainable evidence for a smaller force production by HMM with yeast than with muscle actin could be obtained by examining their sliding over "unprocessed" HMM. In such HMM, the "damaged" heads were not removed by the standard pelleting of HMM and actin in the presence of MgATP. While most of the muscle actin filaments still moved reasonably well over such HMM, despite the intrinsic load generated by the damaged heads, yeast actin filaments normally did not move in such assays. A quantitative comparison of the force generated by HMM with yeast and muscle actins was obtained by monitoring their motion at different molar ratios of NEM−HMM to HMM, i.e., in the presence of different external loads in the motility assays. This method, which has been used in previous studies (Haeberle, 1994; Haeberle & Hemrick, 1995; Miller et al., 1996a) and in the companion paper (Miller et al., 1996b), provides an estimate of relative forces developed

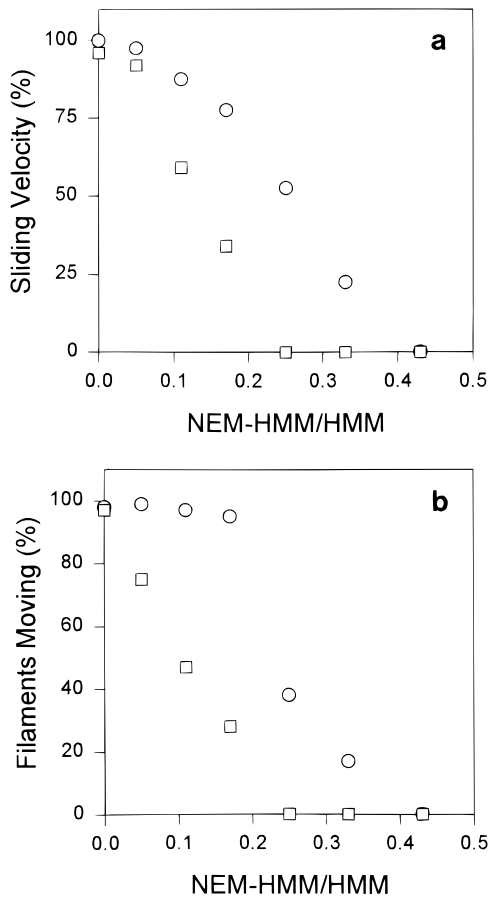


FIGURE 5: *In vitro* motility of yeast and muscle actins in the presence of external load. (a) Relative sliding velocities of yeast actin (□) and and α-actin (○) in the *in vitro* motility assays as a function of the molar ratio of NEM−HMM/HMM applied to the assay surface. Assays were carried out with a 0.25 mg/mL total HMM concentration applied to the cover slip, and the movement was initiated in the assay buffer [1 mM ATP, 25 mM MOPS (pH 7.4), 25 mM KCl, 1.0 mM EGTA, 4.0 mM $MgCl_2$, and 10 mM dithiothreitol]. One hundred percent sliding velocity refers to 3.1 μm/s. The standard deviation of mean values was between 0.1 and 0.3 μm/s. (b) The percentages of yeast (□) and α-actin (○) filaments moving were determined in the same motility assays and at the same ratios of NEM−HMM/HMM as in panel a. At least 100 filaments were analyzed for each sample.

by the compared systems. As shown in Figure 5a, the mean sliding velocity of yeast actin decreased faster than that of α-actin with an increase in the molar ratio of NEM−HMM/HMM. In parallel, the fraction of yeast actin filaments moving against load was smaller than that of α-actin (Figure 5b). The yeast and muscle actin sliding was stopped at molar ratios of NEM−HMM/HMM of 0.25 and 0.43, respectively. This corresponds to an approximate ratio of relative forces of 1.0/1.7 for the yeast and muscle actins.

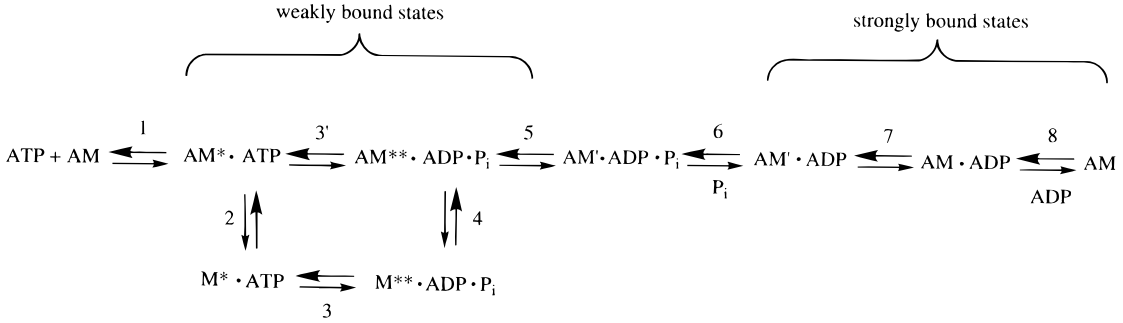
DISCUSSION

Actin Polymerization and Structural Considerations. The evidence for some structural differences between yeast and muscle actin filaments includes (i) kinetic arguments for altered nucleation and for the fragmentation of yeast actin filaments (Buzan & Frieden, 1996), (ii) the observation of such fragmentation in the *in vitro* motility assays (this study), (iii) electron microscopy indications of less extensive contacts between the two long-pitch helical strands in yeast than in muscle actin filaments (Orlova et al., 1996), and (iv) decreased binding of phalloidin to yeast actin (DeLaCruz & Pollard, 1996). This study shows that the faster polymerization of yeast than of muscle Ca^{2+}−G-actin by $MgCl_2$ (Buzan & Frieden, 1996) is also caused by conformational differences between these actins, in particular within the nucleotide cleft region. Nucleotide exchange was found to proceed at a 5-fold faster rate in wild type yeast actin than in α-skeletal Ca^{2+}−G-actin (Miller et al., 1995). If this result is taken to imply a more open nucleotide cleft in the yeast than in muscle Ca^{2+}−G-actin, then the exchange of Ca^{2+} with Mg^{2+} would be facilitated and, thus, the nucleation time for the Mg^{2+}-induced polymerization should be shortened in yeast actin. This was verified by showing that the exchange of Ca^{2+} with Mg^{2+} in G-actin decreased the difference between the rates of Mg^{2+}-induced polymerization of yeast and muscle actins.

More striking is the inhibition of the Mg^{2+}-induced polymerization of yeast actin by KCl. High critical concentrations for the polymerization of yeast Ca^{2+}−G-actin by 0.1 M KCl were reported before (Greer & Scheckman, 1982). However, these findings were attributed to the presence of some minor Ca^{2+}-sensitive contaminant in actin preparations since yeast actin purified by an alternative sequence of chromatographic steps appeared to polymerize well with the addition of 0.1 M KCl (Nefsky & Bretscher, 1992). Clearly, yeast actin purified by DNaseI affinity chromatography does not polymerize with the addition of KCl, despite its "well-behaved" polymerization by Mg^{2+} and Ca^{2+}. The effect of KCl on yeast actin cannot be ascribed to a Ca^{2+}-sensitive factor; the inhibition of Mg^{2+}-induced polymerization of actin by KCl does not support such a possibility. Although the previous observations (Nefsky & Bretscher, 1992) remain unexplained, it is interesting to compare the polymerization of yeast actin with that of loop 38−52-cleaved muscle actin (Schwyter et al., 1989; Khaitlina et al., 1993). The impaired ability of the cleaved loop to form intermolecular contacts is corrected by Mg^{2+} and to a lesser extent by KCl. In yeast actin, the binding of monovalent cations (instead of Mg^{2+} or Ca^{2+}) to several weak affinity sites does not prime G-actin for polymerization. It is attractive to speculate that this behavior of the yeast actin and cleaved muscle actin is related to a decreased stability of the intermolecular contacts of loop 38−52 as compared to that of the intact, uncleaved muscle actin. This aspect of yeast actin filament structure is revealed through their digestion by subtilisin. Other factors which may contribute to the stability of yeast being lower than that of muscle actin filaments are yet to be determined. The limited tests of the C terminus environment and Cys-374 reactivity did not reveal any large differences between the yeast and muscle actin in this region. The different enhancement of pyrenyl actin fluorescence upon polymerization of the two actins may have its origin in local differences, including perhaps such factors as the orientation of the dye. Similar quenching of pyrenyl F-actin fluorescence by S1 for both actins is consistent with such a possibility.

Functional Properties of Yeast Actin. Earlier studies (Miller et al., 1995, 1996a) established that the strong (rigor) binding of S1 to yeast actin filaments ($K_a = 2 \times 10^6$ M^{-1}) is decreased relative to that measured for muscle actin ($K_a = 2 \times 10^7$ M^{-1}). The weak binding of S1 to actin, in the presence of MgATP, is the same for both actins, as is also the K_m value for the acto-S1 ATPase activity (Cook et al., 1993). The V_{max}, which depends on the isomerization

Scheme 1



between the A·M**ADP·P_i and A·M′·ADP·P_i actomyosin complexes in the cross-bridge cycle [Scheme 1, adopted from Ma and Taylor (1994)], is much higher with muscle than with yeast actin (Cook et al., 1993). The results of this study point to the functional consequences of such differences. Clearly, HMM generates less force with wild type yeast actin than with α-actin. It is assumed, in analogy to the companion study (Miller et al., 1996b), that the smaller force results from a decrease in the number of force-generating cross-bridges. Although it is yet to be tested in unitary force measurements, this assumption is supported by equal sliding velocities of yeast and muscle actins at saturating HMM concentrations and the dependence of their motilities on the HMM concentration.

In the absence of a detailed kinetic analysis of the myosin cross-bridge cycle with yeast actin, it is difficult to assess how much the lower V_{max} and a reduced strong binding of S1 contribute to the decrease in the force generated with yeast actin. The comparison of wild type actin with the 4Ac mutant actin (Miller et al., 1996b), which differ only in the V_{max} value of the acto-S1 ATPase but not in their strong or weak binding affinities for S1 (Cook et al., 1993), is helpful. Such a comparison shows that the difference in the isomerization between the A·M**ADP·P_i and A·M′·ADP·P_i complexes (Scheme 1) may be sufficient to yield a greater force with the 4Ac than with wild type actin, at a ratio of 1.3/1.0 with 4Ac/wild type actins, presumably due to an increased flux of cross-bridges into the force-generating states of the cycle (Miller et al., 1996b). Yet, the 4Ac actin, with an α-actin-like N-terminal cluster of acidic residues (Ac-MDEDE...), still underperforms α-actin in strong S1 binding (Miller et al., 1996b), the V_{max} of acto-S1 ATPase (Cook et al., 1993), and the force generated in motility experiments (with force ratios of 1.7/1 for α-actin/wild type yeast actin; this work). Thus, it is not clear yet whether the difference in the force generated by HMM with 4Ac actin and muscle α-actin should be ascribed to the difference in the isomerization step (between A·M**ADP·P_i and A·M′·ADP·P_i), the strong binding of S1, or perhaps other factors. The elucidation of this issue will depend on the ability to selectively change one of these two aspects of actomyosin interaction. Clearly, also, kinetic rates for actomyosin interactions will be needed for the simulation of the cross-bridge cycle (in Scheme 1) with yeast actin.

It may be concluded that the functional differences between yeast and muscle actins do not complicate the interpretation of yeast mutant actin experiments, but they actually contribute to the analysis of the mechanism of cross-bridge action. Finally, on a practical note, it should be emphasized that *in vitro* motility experiments, in much the same way as actomyosin ATPase measurements, must be carried out over a range of protein (HMM) concentrations (and load conditions) to resolve between altered properties of the actomyosin system.

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