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Actin from *Saccharomyces cerevisiae*

CHRIS GREER† AND RANDY SCHEKMAN*

Department of Biochemistry, University of California, Berkeley, California 94720

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Inhibition of DNase I activity has been used as an assay to purify actin from *Saccharomyces cerevisiae* (yeast actin). The final fraction, obtained after a 300-fold purification, is ~97% pure as judged by sodium dodecyl sulfate-gel electrophoresis. Like rabbit skeletal muscle actin, yeast actin has a molecular weight of about 43,000, forms 7-nm-diameter filaments when polymerization is induced by KCl or Mg^{2+}, and can be decorated with a proteolytic fragment of muscle myosin (heavy meromyosin). Although heavy meromyosin ATPase activity is stimulated by rabbit muscle and yeast actins to approximately the same V_{max} (2 mmol of P_i per min per μmol of heavy meromyosin), half-maximal activation (K_{app}) is obtained with 14 μM muscle actin, but requires approximately 135 μM yeast actin. This difference suggests a low affinity of yeast actin for muscle myosin. Yeast and muscle filamentous actin respond similarly to cytochalasin and phalloidin, although the drugs have no effect on *S. cerevisiae* cell growth.

Microfilaments composed of actin participate in many motile processes. Actin and myosin from a number of eucaryotic cell types have been examined, and their biochemical properties are similar (17). The conserved nature of these proteins suggests that microfilament-mediated motile processes in eucaryotic cells share common mechanisms. Because of the variety of functions that actin fulfills in nonmuscle eucaryotic cells, it has been difficult to ascribe biological roles to the biochemical properties of the purified protein. An organism such as *Saccharomyces cerevisiae*, with a limited range of motile functions, may be more suitable for making such a biological-biochemical connection. Actin in *S. cerevisiae* (yeast actin) might participate in chromosome and organelle movement without additional roles in cell movement and shape determination.

Our interest has focused on the secretory process in *S. cerevisiae*. Circumstantial evidence in other systems has suggested a role for microfilaments in movement and discharge of secretory granules. Cytochalasin B, a drug that destabilizes microfilaments, causes secretory granules to accumulate in maize root tip cells and fertilized *Fucus* embryos (3, 24) and blocks thyroid secretion (41). We have identified 23 genes that are required for secretion and bud growth in *S. cerevisiae* (28). Mutant alleles of these genes confer thermosensitive growth and cause the accumulation of secretory organelles and secretory glycoproteins. Mutations in the single yeast actin gene (9, 27) might produce the secretory mutant phenotype.

We have devised an actin purification scheme by using inhibition of DNase I as an assay (20). Previous yeast actin isolation procedures have demonstrated the selective nature of yeast actin-DNase I interaction, but the purification procedures yielded impure or inactive preparations (18, 39). The procedure described in this report has allowed an investigation of the functional properties of the purified protein. This information may provide a basis for examining the role of actin in the secretory process.

MATERIALS AND METHODS

Materials. DEAE-cellulose DE-52 was obtained from Whatman Ltd., Clifton, N.J. DEAE-Sephadex A-50, Sephadex G-150, Octyl-Sepharose C1-4B, and carrier ampholytes were from Pharmacia Fine Chemicals, Inc., Piscataway, N.J. Enzyme-grade ammonium sulfate was from Schwarz/Mann, Orangeburg, N.Y. Cytochalasin B was from Aldrich Chemical Co., Milwaukee, Wis. Phalloidin was from Boehringer Mannheim Corp., New York, N.Y. All other chemicals were analytical reagent grade unless specified.

Strain and cell growth. *S. cerevisiae pep4*, a mutant obtained from E. Jones, is a derivative of the standard wild-type strain X2180 that has reduced levels of the three major vacuolar protease activities (13). Cells were grown in YPD medium (1% yeast extract, 2% peptone, and 2% glucose) at 30°C to a density of 10^8 cells per ml. Cells were harvested by centrifugation, washed once with distilled water, and stored at −15°C.

Buffer. Buffer D (11) contained 0.5 mM ATP, 0.75 mM β-mercaptoethanol, 0.1 mM $CaCl_2$, and 10 mM imidazole-hydrochloride (pH 7.5). NaN_3 (1 mM) was added to dialysis buffers.

† Current address: Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Muscle proteins. Rabbit back and leg fast muscle was used to prepare actin by the method of Rees and Young (33), except that a Sephadex G-150 column (2.7 by 45 cm) equilibrated with buffer D was used in the final step. Myosin prepared by the method of Tonomura et al. (35) was stored at −15°C in 50% glycerol–0.6 M KCl–10 mM imidazole-hydrochloride (pH 7.5). Heavy meromyosin (HMM) was prepared by the method of Eisenberg and Moos (8) from myosin which had been dialyzed against 0.6 M KCl–20 mM imidazole-hydrochloride (pH 6.8).

Chromatography materials. DEAE chromatography materials were saturated with ATP to prevent denaturation of actin (11). DEAE-cellulose (1 liter, wet volume) in buffer D plus 0.1 M KCl or DEAE-Sephadex in buffer D plus 0.05 M KCl was treated with 4 to 5 g of ATP (grade II; Sigma Chemical Co., St. Louis, Mo.). The ATP-saturated material was then washed in a column until the pH and absorbancy at 260 nm of the effluent were the same as those of buffer D. Octyl-Sepharose was recycled by washing sequentially with 10 volumes each of 1% Triton X-100 in water, 95% ethanol, 50% ethanol, 10% ethanol, and distilled water. Washing was continued in a column with 5 volumes each of distilled water and buffer, followed finally by buffer D plus 0.4 M KCl.

Actin concentration. Dilute samples of yeast actin were concentrated in an Amicon 65-ml ultrafiltration cell with a PM-10 membrane. A flow rate of 0.5 ml/min was obtained with nitrogen at 25 to 30 lb/in^2. Concentration to greater than 1 mg of protein per ml by this method resulted in reduced recovery of actin, possibly due to binding of actin to the ultrafiltration membrane. More concentrated solutions were obtained by dialysis against Ficoll type 400 powder (Sigma).

Viscometry. Viscometry was done at 26°C in an Ostwald-type viscometer with a buffer flow time of 77 s. The relative viscosity is the flow time of the sample divided by the buffer flow time. The specific viscosity is the relative viscosity minus one. The reduced viscosity is the specific viscosity divided by the protein concentration in milligrams per milliliter.

Gel electrophoresis. Sodium dodecyl sulfate (SDS)-gel electrophoresis was performed on 10% acrylamide slabs as described by Ames (1), except that the running buffer contained 0.38 M Tris-hydrochloride (pH 8.1). Gels were fixed and stained for 1 h in 0.05% Coomassie blue–10% acetic acid–25% isopropanol and then destained in 10% acetic acid. Isoelectric focusing in 2.5- by 150-mm polyacrylamide tube gels was done by the method of O'Farrell (29) with Pharmacia pH 4 to 6.5 carrier ampholytes. Gels were electrophoresed at 400 V for 20 h and fixed for 40 min in 20% trichloroacetic acid. Detergent and ampholytes were removed by soaking the gels for 3 h in 35% ethanol–10% acetic acid (ethanol-acetate). Gels were stained in ethanol-acetate with 0.02% Coomassie blue and destained for 6 to 8 h in ethanol-acetate.

Electron microscopy. Samples were examined in a Phillips EM301 electron microscope at an accelerating voltage of 80 kV. Specimen grids with carbon-stabilized Parlodion films were glow discharged before use. Microfilaments were formed from actin (0.07 mg/ml in buffer D) by incubation with 1.5 mM $MgCl_2$ for 30 min at 22°C. A polymerized sample was diluted 20-fold with the same buffer, 5 μl was placed quickly on a sample grid, and the excess solution was drawn off with a tissue. The grid was washed with a solution of cytochrome *c* and stained with uranyl acetate as described by Nachmias et al. (26). HMM-decorated filaments were obtained by mixing HMM (0.8 mg/ml) and actin (0.15 mg/ml) in buffer D with 1.5 mM $MgCl_2$ and lacking ATP. The sample was incubated and processed as described above.

Protein and ATP estimations. Protein was measured by the method of Bradford (2) with bovine serum albumin as a standard. Protein standards were prepared by using an E_{280} for bovine serum albumin of 0.667 ml · mg^{-1} · cm^{-1} and for DNase I of 1.23 ml · mg^{-1} · cm^{-1}. ATP (grade I; Sigma) substrate solutions for ATPase assays were made by using an E_{259} of 15.3 mM^{-1} · cm^{-1}.

DNase assay. Actin was measured by inhibition of DNase activity (20). Calf thymus DNA (type I; Sigma) was suspended in sterile water (0.33 mg/ml) and dissolved at 22°C overnight. After $MgSO_4$ and $CaCl_2$ were added to 8 mM and Tris-hydrochloride was added to 50 mM, the pH was adjusted to 7.5 with 1 N HCl. A stock solution of DNase I (1 mg/ml, type DN-C1; Sigma) was prepared in 50 mM Tris-hydrochloride (pH 7.5). Both stock solutions were stored at −15°C. The assay contained 20 μl of DNase I stock diluted 100-fold, the actin sample, 20 μl of buffer D (including the amount in actin sample), and 50 mM Tris-hydrochloride (pH 7.5) to a final volume of 0.5 ml. This mixture was incubated for 4 to 6 min at 24°C to depolymerize any filamentous actin present (14). The DNA substrate (0.5 ml) was added, and the change in absorbancy at 260 nm was measured in a recording spectrophotometer. One unit of DNase activity produces an increase in absorbancy at 260 nm of 0.001/min; 1 U of actin decreases the specific activity of DNase by 10^6 U/mg. The inhibition of DNase activity, up to 70%, was proportional to the amount of actin.

HMM ATPase assay. Assays (0.2 ml) contained 2.5 mM $MgCl_2$, 0.05 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid, 10 mM imidazole-hydrochloride (pH 7.0), and 0.25 to 0.3 μM HMM. Actin samples were polymerized with 5 mM $MgCl_2$. Reactions were started by the addition of 2 mM ATP and incubated for 5 to 30 min at 30°C to allow hydrolysis of 5 to 25% of the ATP. Trichloroacetic acid (0.8 ml, 20%) was added to stop the reactions, and precipitated protein was removed by centrifugation at 27,000 × *g* for 10 min. P_i in the supernatant solution was measured by the method of Marsh (23). A background value, obtained without HMM, was subtracted from all assays. The activity of HMM alone (13 μmol of P_i per min per μmol of HMM) was subtracted from the activity in the presence of actin to obtain values for ATPase activation. The background value for actin alone was variable (0.02 to 2.2 μmol of P_i per min per μmol of actin), but much lower than the activity of HMM alone.

RESULTS

Purification of yeast actin. All procedures were done at 4°C.

(i) Crude extract. Cells (375 g, wet weight) were suspended in 375 ml of buffer D, and phenylmethylsulfonyl fluoride was added to a final concentration of 1 mM. Cells were lysed by four passes through a Manton-Gaulin homoge-

nizer at 9,000 lb/in^2. During this procedure the extract was cooled by the addition of frozen buffer. The lysate was centrifuged at 11,000 × *g* for 20 min, and phenylmethylsulfonyl fluoride (1 mM increment) was added to the supernatant fraction (fraction I). No significant amount of actin was detected in the sedimented fraction by DNase inhibition or by SDS gel electrophoresis (data not shown).

(ii) DEAE-cellulose chromatography. Fraction I was applied to a DEAE-cellulose column (7 by 45 cm; 10 mg of protein per ml bed volume) followed by 1.5 column volumes of buffer D. An 11-liter linear gradient from 0.1 to 0.4 M KCl in buffer D was applied, and the column was eluted at 0.6 liter per h. Actin eluted between 0.19 and 0.23 M KCl. The pooled material (fraction II) contained 80% of the starting DNase inhibition activity.

(iii) Ammonium sulfate fractionation. Solid ammonium sulfate was mixed with fraction II to 20% saturation (with respect to 0°C). After 15 min the solution was centrifuged at 23,000 × *g* for 20 min, and the supernatant fraction was mixed with more ammonium sulfate to 65% saturation. The precipitate was collected by centrifugation as described above, and the pellet was suspended by homogenization in 100 ml of buffer D containing ammonium sulfate to 45% saturation. The 45% precipitate was centrifuged at 27,000 × *g* for 15 min, and the pellet was extracted with 50 ml of buffer D containing ammonium sulfate to 30% saturation. After centrifugation, the previous extraction was repeated, and the 30% soluble fractions were pooled and dialyzed twice for 8 h against 2 liters of buffer D. The dialyzed material (fraction III) had 80% of the activity from fraction II and could be stored at 4°C for several days. The first steps of the purification procedure were completed within 24 h as the actin was unstable before fraction III.

(iv) DEAE-Sephadex chromatography. Fraction III was applied to a DEAE-Sephadex column (4 by 16.5 cm) followed by 400 ml of buffer D plus 0.05 M KCl and 400 ml of buffer D plus 0.2 M KCl. An 800-ml linear gradient from 0.2 to 0.4 M KCl in buffer D was applied, and the column was eluted at 60 ml/h. Actin eluted between 0.29 and 0.34 M KCl. The pooled material (fraction IV) contained 74% of the activity from fraction III.

(v) Octyl-Sepharose. Fraction IV was adjusted to 0.4 M KCl and concentrated by ultrafiltration to 0.8 mg of protein per ml. The concentrated fraction was applied at 20 ml/h to an octyl-Sepharose column (2.7 by 16 cm) which was then washed at 50 ml/h with 400 ml of buffer D–0.4 M KCl. The column material was transferred into tubing and dialyzed against buffer D until the conductivity of the sample was equal to that of the buffer. Actin, desorbed from the octyl-Sepharose, was recovered by returning the dialysate to a column which was washed with buffer D. Fractions of the eluate containing most of the activity (70% of fraction IV) were pooled and concentrated by ultrafiltration and treatment with solid Ficoll to a final volume of 4 ml (fraction V). Attempts to desorb actin from octyl-Sepharose by elution with a large volume of buffer D were unsuccessful. Standard elution, which generated either a slow or rapid decrease in ionic strength, resulted in dilution below a protein concentration necessary to maintain actin activity.

(vi) Gel filtration. Fraction V was applied to a Sephadex G-150 column (2.7 by 48 cm, overlaid with 1.5 cm of Sephadex G-25) equilibrated in buffer D. Fractions (3 ml) were collected at 7.5 ml/h. Constant specific activity was seen across the single protein peak. The pooled (fraction VI) material contained 60% of the activity from fraction V. The elution profile for purified rabbit muscle actin was identical.

A summary of the purification is shown in Table 1. The DNase inhibition specific activity of purified yeast actin was about 20% greater than that of rabbit actin (1,440 versus 1,210 U/mg; Fig. 1). The final fraction was 97% pure as judged by scanning SDS gels of the final fraction in a Gelman densitometer with an integrating recorder.

Gel electrophoresis. A 43-kilodalton (43K) protein (as judged by SDS-gel electrophoresis) was enriched at each stage of the purification procedure (Fig. 2). No difference in electrophoretic mobility between yeast and rabbit muscle actin was detected in our gel system. Extrapolating the linear portion of the inhibition curve in Fig. 2 to 100% inhibition gives a ratio of 1.08 mol of yeast actin to 1 mol of DNase I (assuming a molecular weight of 43,000 for yeast actin).

TABLE 1. Summary of purification of yeast actin

Fraction	Total actin (U)	Sp act (U/mg)	% Recovery	Purification (fold)
I. Crude extract	99,900	4.7		
II. DEAE-cellulose	78,700	118	79	25
III. Ammonium sulfate	66,015	200	66	43
IV. DEAE-Sephadex	49,020	538	49	114
V. Octyl-Sepharose	34,230	1,085	34	231
VI. Sephadex G150	20,890	1,440	20	306

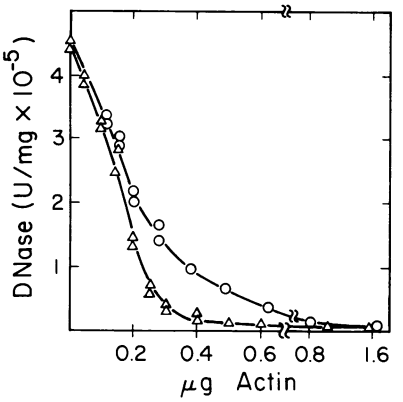

FIG. 1. Actin inhibition of DNase I. Various amounts of yeast actin (△) or rabbit actin (○) were added to a standard DNase assay containing 0.2 μg of DNase I as described in the text.

The final fraction contained ~3% of a 36K to 38K protein. This minor species bound weakly to a DNase I affinity column (20) and could be eluted with 1 M urea, whereas most of the actin required 3 M urea for elution (data not shown). Unfortunately, this was not a useful purification step because urea-denatured actin cannot be renatured to a completely active species (39). Other purification steps, such as polymerization and sedimentation of filamentous actin, did not resolve the contaminant. Occasional preparations had a higher level of this contaminant, which resulted in a lower final specific activity.

The isoelectric points of rabbit muscle and yeast actins were compared by isoelectric focusing in polyacrylamide tube gels that contained urea and Nonidet P-40. The single yeast actin species was about 0.1 pH unit more basic than rabbit actin.

Electron microscopy. Yeast actin polymerized in 1.5 mM $MgCl_2$ was examined by electron microscopy. The filaments (Fig. 3A) were indistinguishable from muscle actin, having a diameter of 7.0 ± 0.3 nm (n = 10). Muscle myosin interaction with actin filaments can be assessed by electron microscopy. A tryptic fragment of muscle myosin, HMM, binds to actin filaments in the absence of ATP to form arrowhead-shaped structures (15). Polymerized yeast actin showed a pattern of decoration by HMM (Fig. 3B), with arrowheads revealing the polarity of the filaments. The arrowheads had a maximum width of 20 nm and a repeat distance of 37.8 ± 2.2 nm (n = 10). The latter value, which is similar for other actins, is a measure of the helix crossover distance of the actin filament (25).

Actomyosin ATPase. Actins from muscle and many nonmuscle sources will activate muscle myosin ATPase activity (34). Actins from diverse sources appear to activate myosin to the same V_{max}, but with different amounts required for half-maximal activation (K_{app}) (10, 11, 42). Figure 4 shows the results of HMM ATPase

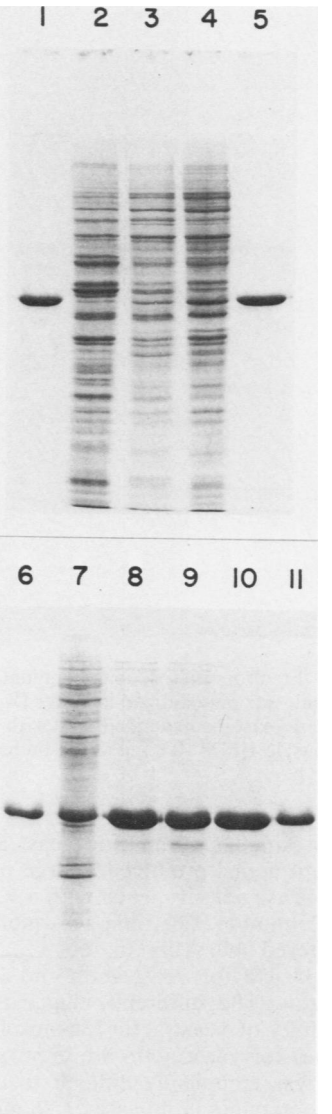

FIG. 2. Analysis of purification fractions by polyacrylamide gel electrophoresis. Samples of fractions obtained in the purification of yeast actin were electrophoresed on a 10% polyacrylamide slab gel containing 0.1% SDS. Rabbit actin (2 μg) was applied in lanes 1, 5, 6, and 11. A constant amount of protein (5 μg) was applied in each of the following lanes: 2, fraction I; 3, fraction II; 4, fraction III; 7, fraction IV; 8, octyl-Sepharose eluate; 9, fraction V (concentrated octyl-Sepharose eluate); 10, fraction VI.

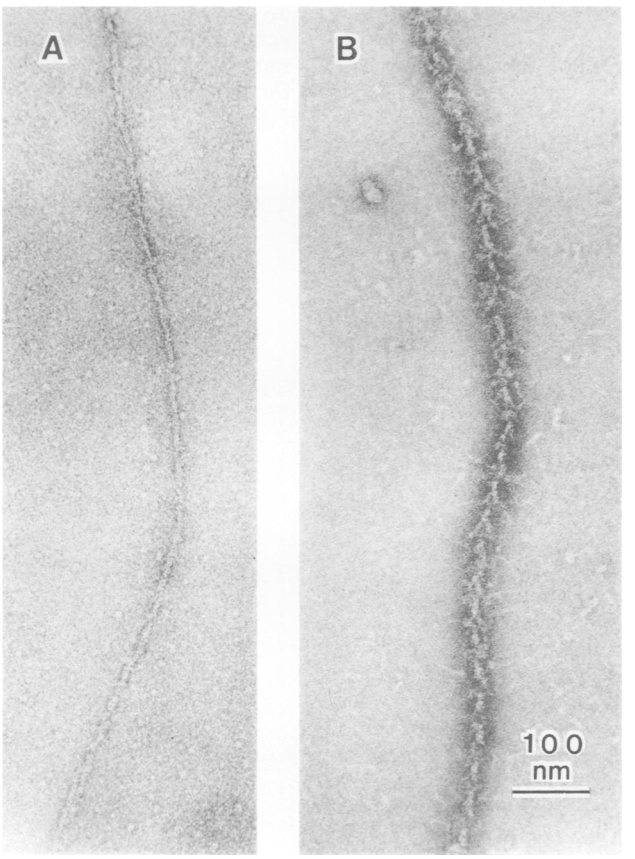

FIG. 3. Electron microscopic examination of yeast actin filaments. (A) A single yeast actin filament. Actin (0.07 mg//ml) was polymerized in buffer D–1.5 mM $MgCl_2$ for 30 min at 22°C and then diluted 20-fold in the same buffer (B). A yeast filament decorated with muscle HMM, a tryptic subfragment of myosin. Yeast actin (0.15 mg/ml) and muscle HMM (0.8 mg/ml) in buffer D lacking ATP were polymerized and incubated as in (A).

activation kinetics comparing yeast and rabbit actin. Both actins produced a large increase in HMM ATPase activity, each with a V_{max} on the order of 2 mmol of P_i per min per μmol of HMM. They differed markedly in the K_{app}: approximately 135 μM for yeast actin and 14 μM for rabbit actin. The difference suggests a much lower affinity of yeast actin for muscle myosin. The values for yeast actin are approximate because it was technically difficult to use higher than K_{app} amounts in an assay (~6 mg/ml).

Polymerization measured by viscometry. The ability of yeast and rabbit muscle actin preparations to polymerize were compared by viscometry (Fig. 5). At actin concentrations of 0.35 to 0.5 mg/ml, both actins polymerized at Mg^{2+} concentrations above 0.6 mM and KCl concentrations above 10 mM. The presence of 0.1 mM Ca^{2+} caused a small increase in the concentration of Mg^{2+} required for polymerization and increased the reduced viscosity of both actins at 2 mM Mg^{2+}. Whereas 0.1 mM Ca^{2+} had little effect on muscle actin polymerized in KCl (not shown), an increase in the viscosity of yeast actin in KCl plus Ca^{2+} was seen only at higher yeast actin concentrations (accompanying paper [12]).

The critical concentration (C_∞) is the minimum concentration for actin polymerization and is equal to the steady-state concentration of actin monomer in the presence of ATP (30). C_∞ varies with the source of actin and the conditions of polymerization. Conditions sufficient for polymerization, chosen from the previous experiment, were used with varied concentrations of actin to determine C_∞, which was measured by extrapolating to zero viscosity. The C_∞ for rabbit actin in 100 mM KCl was 0.04 mg/ml and in 2 mM $MgCl_2$ was 0.02 mg/ml; the presence of 0.1 mM $CaCl_2$ had no effect on these values (Fig. 6). The C_∞ for yeast actin in 100 mM KCl was 0.04 mg/ml and in 2 mM $MgCl_2$ was 0.03 mg/ml.

Effect of cytochalasin and phalloidin. Viscome-

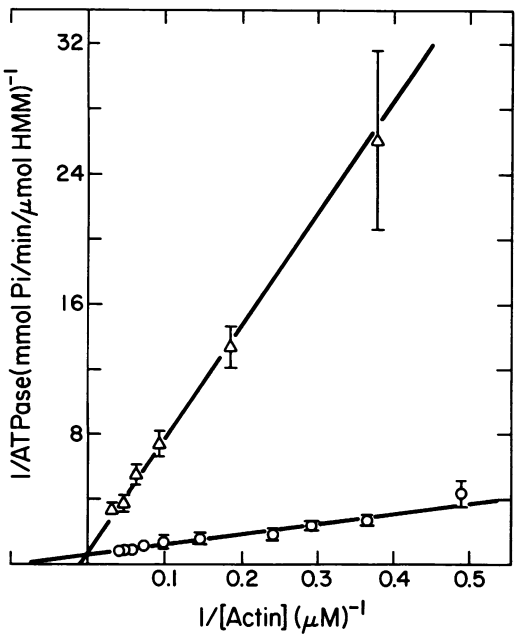

FIG. 4. Yeast actin activation of muscle myosin ATPase. Double-reciprocal plot of the effect of yeast actin (△) and rabbit muscle actin (○) on the Mg^{2+}-ATPase activity of rabbit muscle HMM. ATPase activity was measured at 30°C in 2.5 mM $MgCl_2$–0.05 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid–2 mM ATP–10 m*M* imidazole-hydrochloride (pH 7.0). Actin samples were polymerized in 5 mM $MgCl_2$. Values for ATPase activation were obtained by subtracting the ATPase activity of both the HMM and actin alone from the activity of the mixture.

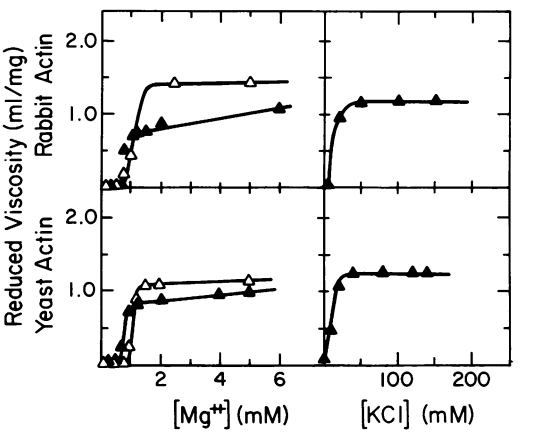

FIG. 5. Effect of KCl and Mg^{2+} concentration on polymerization of yeast and rabbit actins. The ability to polymerize in response to KCl or Mg^{2+} was examined by measuring the viscosity of actin solutions at 26°C in an Ostwald-type viscometer. Actin samples (0.35 to 0.5 mg/ml) in buffer D (△) or buffer D plus 0.2 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid (▲) were incubated for 3 h at room temperature after the addition of KCl or Mg^{2+}. The upper panels are the results with rabbit muscle actin, the lower panels are the results with yeast actin.

try was also used to compare the effects of cytochalasin B and phalloidin on rabbit muscle and yeast actins. Cytochalasin B causes a decrease in the viscosity of muscle actin filaments (21). Phalloidin binds to actin and stabilizes filaments to depolymerizing agents such as KI and DNase I (40). The effects of these two drugs were assessed with actins polymerized in 2 mM $MgCl_2$ (Table 2). Cytochalasin B decreased the viscosity of both yeast and rabbit actin filaments more than 50%. Phalloidin protected both types of filaments from KI-induced depolymerization. These drugs had no effect on the growth rate of yeast cells, perhaps because of the relative impermeability of the yeast cell surface.

DISCUSSION

Our procedure for the purification of yeast actin is based in part on the method of Gordon et al. (11). Additional purification steps were selected which minimized a reliance on functional properties of actin. The nonselective nature of this procedure and the high yield in each step

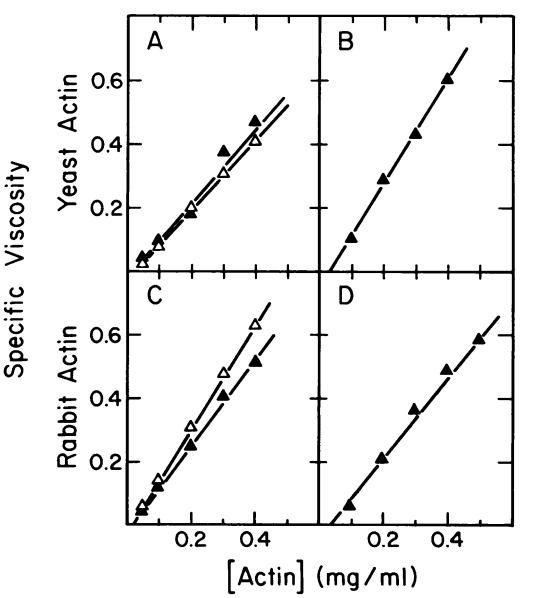

FIG. 6. Effect of actin concentration on polymerization. The minimum actin concentration for polymerization in 2 mM $MgCl_2$ (A and C) or 100 mM KCl (B and D) was determined by viscometry. Yeast actin (A and B) or rabbit muscle actin (C and D) in buffer D (△) or buffer D plus 0.2 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid (▲) were incubated for 3 h at room temperature after the addition of KCl or Mg^{2+}. Viscosity was measured at 26°C.

TABLE 2. Effects of cytochalasin B and phalloidin on the specific viscosity of yeast and muscle filamentous actin[a]

Actin	Initial specific viscosity	Specific viscosity after treatment with:			
		3% DMSO	400 μM cytochalasin	0.6 M KI	15 μM phalloidin, 0.6 M KI
Yeast	0.243	0.250	0.090	0.022	0.231
Rabbit	0.230	0.237	0.079	0.043	0.188

[a] Yeast and rabbit muscle actin samples (0.22 mg/ml in buffer D) were incubated 3 h at room temperature after the addition of 2 mM $MgCl_2$. Viscosity was measured at 26°C. Samples were further incubated for 1 h at 26°C after making the appropriate addition. Cytochalasin B was added in dimethyl sulfoxide (DMSO) to a final DMSO concentration of 3% (vol/vol).

suggest that the purified material is representative of the cellular pool. These results, taken together with the evidence of a single actin gene in *S. cerevisiae* (9, 27), suggest one major species of functional actin.

The binding of yeast actin to octyl-Sepharose may involve polymerization. Efficient binding during purification requires slow adsorption and high protein concentration (>0.6 mg/ml). Hydrophobic interaction also is seen with muscle filamentous actin, which binds to polystyrene beads, whereas globular actin does not (31). Thus, although only polymerization-competent actin may be recovered from the octyl-Sepharose step, the yield of DNase inhibition activity is 70 to 90%.

The final fraction of purified actin contains a small amount (3%) of a 36K to 38K protein. This minor species could represent a contaminant or an actin fragment. Various amounts of proteins in this molecular weight range have been detected in other actin preparations. A 38K protein is found in crystallized bovine spleen actin and profilin (5). Inhibition of protease activity by phenylmethylsulfonyl fluoride added during purification reduces the amount of this species (6). Limited trypsin treatment of muscle actin produces a 34K form (16). We have been unable to completely resolve this minor species and retain functional actin, although separation of inactive actin can be achieved in urea or SDS (39).

The major properties of rabbit muscle and yeast actins are similar. Both have molecular masses of about 43K, both polymerize to form 7-nm-diameter filaments, both activate HMM ATPase to the same V_{max}, and both are decorated by HMM to form characteristic arrowhead structures. The slopes of the critical concentration curves (Fig. 6) and the values for maximum reduced viscosity at high ionic strength (Fig. 5) are similar for the two actins. This suggests that the amount and average length of the filaments formed in the two preparations are the same, and that most of the yeast actin is capable of polymerization. The similarity in function among actins is reflected in a highly conserved amino acid sequence. A comparison of sequence homology among actins from five sources is shown in Table 3. Although actins have been classified as muscle or nonmuscle types based on the degree of sequence homology (22, 38), yeast actin does not appear to fall into either class.

One measure of the difference between rabbit muscle and yeast actin is the value of K_{app} for activation of muscle HMM ATPase activity (Fig. 4). HMM retains both the ATPase and actin binding sites of myosin. Careful measurements of the ATPase activation kinetics by various actins have shown similar V_{max} and different K_{app} values (10, 11, 42). This behavior is predicted by the refractory state model proposed by Eisenberg and co-workers (34), in which the rate-limiting step in ATP hydrolysis by actomyosin, a transition of myosin from a refractory to a nonrefractory state, is independent of actin. The nonrefractory state rapidly

TABLE 3. Amino acid sequence comparison[a]

Actin source	% Nonhomology		Reference
	Yeast actin	*Physarum* actin	
Physarum polycephalum	11		36
Bovine Brain	13	4	37
Bovine Aorta	14	8	38
Rabbit Skeletal Muscle	14	8	7

[a] The amino acid sequences of two cytoplasmic actins (*P. polycephalum* and bovine brain) and two muscle actins (bovine aorta and rabbit skeletal muscle) were compared. A sequence of 370 amino acids was determined from the nucleotide sequence of the actin gene (9, 27). The amino acid sequences obtained from the studies listed in the table were aligned to give maximum sequence homology. The percent nonhomology is the number of amino acid differences divided by the total number of positions compared (370 for *S. cerevisiae* and 374 for *P. polycephalum*) multiplied by 100.

converts to a tight-binding form which is susceptible to actin binding reflected in the K_{app} value. The large difference in K_{app} for yeast and muscle actins suggests a much lower affinity of yeast actin for muscle myosin. This difference is larger than that observed in comparisons of *Dictyostelium* and *Acanthamoeba* actins with muscle actin (11, 42). The K_{app} for yeast actin (~6 mg/ml) is higher than the intracellular concentration of actin (~0.5 mg/ml) estimated from cell volume and the amount of actin in a crude extract. There may be a yeast myosin with a higher affinity for yeast actin.

Unlike rabbit muscle actin, the KCl-induced viscosity of yeast actin was rapidly and reversibly reduced by low levels of Ca^{2+}. In an accompanying paper we report on the nature of this Ca^{2+} effect (12).

The cellular functions of yeast actin remain to be determined. Although actin may participate in cytokinesis, it probably does not constitute the ring of filaments that lies adjacent to the plasma membrane in the area of the division septum in budding yeast (4). These filaments are 10 nm in diameter instead of the 7-nm filaments observed with purified actin. The 10-nm filaments may be more closely related to intermediate filaments observed in smooth and skeletal muscle (19). Yeast actin mutants that confer conditional growth may provide insight into the cellular function of actin.

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