MOLECULAR AND CELLULAR BIOLOGY, Oct. 1982, p. 1279–1286
0270-7306/82/101279-08$02.00/0

Vol. 2, No. 10

Calcium Control of *Saccharomyces cerevisiae* Actin Assembly

CHRIS GREER† AND RANDY SCHEKMAN*

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

Received 15 March 1982/Accepted 15 June 1982

Low levels of Ca^{2+} dramatically influence the polymerization of *Saccharomyces cerevisiae* actin in KCl. The apparent critical concentration for polymerization (C_∞) increases eightfold in the presence of 0.1 mM Ca^{2+}. This effect is rapidly reversed by the addition of ethylene glycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid or of 0.1 mM Mg^{2+}. Furthermore, the addition of Ca^{2+} to polymerized actin causes a reversible increase in the apparent C_∞. In the presence of Ca^{2+}, at actin concentrations below the apparent C_∞, particles of 15 to 50 nm in diameter are seen instead of filaments. These particles are separated from soluble actin when Ca^{2+}-treated filamentous actin is sedimented at high speed; both the soluble and particulate fractions retain Ca^{2+}-sensitive polymerization. The Ca^{2+} effect is *S. cerevisiae* actin-specific: the C_∞ for rabbit muscle actin is not affected by the presence of Ca^{2+} and *S. cerevisiae* actin. Ca^{2+} may act directly on *S. cerevisiae* actin to control the assembly state in vivo.

Actin microfilaments are a major structural element of the eucaryotic cytoskeleton. A comparison of the requirements for polymerization of actin in vitro with the conditions of ionic strength and actin concentration thought to exist in vivo indicates that nearly all of the actin in cells should be polymerized (6). The dynamic nature of the actin cytoskeleton, however, and the existence of pools of unpolymerized cytoplasmic actin (6) suggest that the polymerization state of actin is regulated. Regulatory features include control of the timing and location of filament formation and destruction, the orientation and length of filaments that are formed, and the aggregation of filaments to form larger structures. A number of factors which affect actin assembly and which are thought to be involved in cytoskeletal organization have been described (for recent reviews, see references 10 and 12). The interaction of many of these factors with actin is affected by Ca^{2+}.

We are interested in the potential function of actin in the secretory process. The study of the secretory process in the yeast *Saccharomyces cerevisiae* has been facilitated by the isolation of thermosensitive mutants which accumulate secretory intermediates at the restrictive temperature (14). Mutations in the actin gene, or genes whose products are involved in cytoskeletal organization, might result in the secretory mutant phenotype. To provide a basis for pursuing this possibility, we have characterized the biochemical properties of *S. cerevisiae* actin (yeast actin). The essential functional property of actin is polymerization; characterization of the polymerization process and identification of control mechanisms are of special interest. We report in an accompanying paper the purification and characterization of yeast actin (7). Although similar in many aspects to other actins, yeast actin is unique in the effect of calcium on KCl-induced polymerization. Low levels of calcium cause a rapid reduction in the high-shear viscosity of filaments formed in KCl. This effect can be reversed by low levels of magnesium or by removing free calcium with ethylene glycol bio(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid (EGTA). This rapid and reversible effect on filament structure may represent a mechanism for the control of actin assembly. We report here on the nature of this Ca^{2+} effect.

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

MATERIALS AND METHODS

Materials and methods were as described in the accompanying paper.

Centrifugation. The effect of Ca^{2+} on the sedimentation of filamentous actin (F-actin) was examined by centrifugation for 12 h at 105,000 × *g* (r_{max}) in a Beckman type 40 rotor at 20°C. Muscle globular actin (G-actin) did not sediment under these conditions.

RESULTS

Ca^{2+} inhibition of polymerization. The KCl-induced polymerization of yeast actin, unlike that of other actins, is sensitive to low levels of Ca^{2+}. In the presence of 0.1 mM $CaCl_2$ no viscosity increase was detected at KCl concentrations up to 0.6 M and actin concentrations up

to 0.4 mg/ml (Fig. 1A). The effect of higher actin concentrations on calcium inhibition of polymerization was measured by viscometry. Yeast actin in buffer D (contains 0.1 mM Ca^{2+}), or the buffer D plus 0.3 mM EGTA, was incubated for 3 h at room temperature after the addition of 0.1 M KCl. Viscosity was measured at 26°C. Excess EGTA (0.3 mM) was then added to samples in buffer D, and the effect on viscosity was measured after 1 h at room temperature. The results are shown in Fig. 1A. The apparent critical concentration (C_∞) for polymerization can be determined by extrapolating to zero viscosity. The slope of the critical concentration curve provides a measure of the number and length distribution of the filaments that are formed. In the absence of Ca^{2+} the C_∞ for yeast actin (0.06 mg/ml) and the slope of the curve were similar to that measured for muscle actin (Fig. 1 and reference 5). In the presence of 0.1 mM Ca^{2+} the C_∞ was increased eightfold to 0.48 mg/ml. The slope of the curve was nearly identical to that obtained in the absence of Ca^{2+}. Subsequent addition of EGTA substantially reversed the effect of Ca^{2+}; the C_∞ was lowered to 0.1 mg/ml while the slope of the curve remained constant.

Ca^{2+}-induced depolymerization. The viscosity of yeast actin filaments formed in 0.1 M KCl is rapidly and reversibly reduced by the addition of low levels of Ca^{2+}. Yeast actin in buffer D, or buffer D plus 0.3 mM EGTA, was incubated for 3 h at room temperature after the addition of 0.1 M KCl. Viscosity was measured at 26°C. Excess Ca^{2+} (0.6 mM) was then added to samples that contained 0.3 mM EGTA, and the effect on viscosity was measured. The results are shown in Fig. 1B. The addition of Ca^{2+} to polymerized actin increased the C_∞ about fivefold to 0.3 mg/ml while the slope of the curve remained constant. The magnitude of the effect of Ca^{2+} on the viscosity of preformed filaments was less than that observed when Ca^{2+} was present during polymerization. Either equilibrium was not reached in this experiment, or Ca^{2+} affects F-actin and G-actin differently.

Mg^{2+} alters the effect of Ca^{2+}. The effect of Mg^{2+} on Ca^{2+} inhibition of polymerization was also examined. Yeast actin in buffer D, or buffer D plus 0.1 mM $MgCl_2$, was incubated for 3 h at room temperature after the addition of 0.1 M KCl. Viscosity was measured at 26°C. Excess EGTA (0.2 mM) was added to samples that contained Mg^{2+}, and the effect on viscosity was measured. The results are shown in Fig. 1C. Mg^{2+} (0.1 mM) lowered the C_∞ for polymerization in the presence of Ca^{2+} to 0.1 mg/ml. Subsequent addition of EGTA had no significant effect on viscosity.

Effect of Ca^{2+} on sedimentation of KCl-induced filaments. Under polymerizing conditions, not all actin is present as F-actin (11, 15). The amount of G-actin in equilibrium with polymer is characteristic of the source of actin and the conditions for polymerization. To test whether the effect of Ca^{2+} on the apparent C_∞ measured by viscometry was due to a shift in equilibrium favoring G-actin, we examined the effect of Ca^{2+} on sedimentation of KCl-induced filaments. Ac-

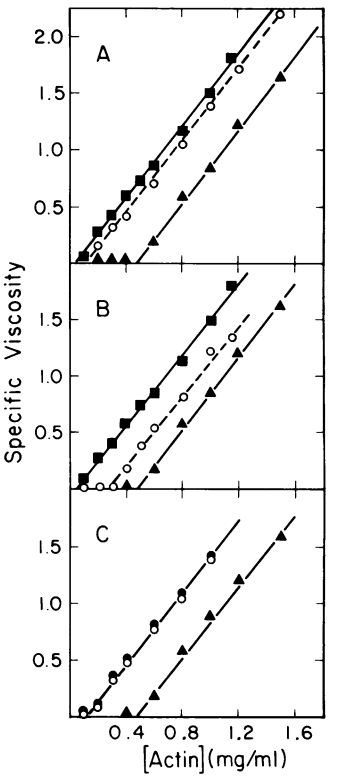

FIG. 1. Effect of Ca^{2+} on the viscosity of yeast actin in KCl. Yeast actin samples in buffer D (▲) or buffer D plus 0.3 mM EGTA (■) were incubated for 3 h at room temperature after the addition of 0.1 M KCl. Viscosity was measured at 26°C. (A) The reversibility of Ca^{2+} inhibition of polymerization in KCl was examined in samples polymerized in buffer D plus 0.1 M KCl (▲). Excess EGTA (0.3 mM) was added to these samples, and viscosity was measured after 1 h at room temperature (○). (B) The effect of adding Ca^{2+} to samples polymerized in the absence of Ca^{2+} (■) was tested. Excess Ca^{2+} (0.6 mM) was added to these samples, and viscosity was measured after 1 h at room temperature (○). (C) The effect of Mg^{2+} on Ca^{2+} inhibition of polymerization was tested. Actin samples in buffer D plus 0.1 mM $MgCl_2$ (●) were incubated for 3 h at room temperature after the addition of 0.1 M KCl. Viscosity was measured at 26°C. Excess EGTA (0.3 mM) was added, and samples were further incubated for 1 h at room temperature before measuring viscosity (○). Longer incubations had no effect on viscosity.

TABLE 1. Effect of Ca^{2+} on sedimentation of actin[a]

Actin sample	0.3 mM EGTA						0.1 mM $CaCl_2$		
	Initial			With excess $CaCl_2$					
	η_{sp}[b]	% Recovery[c]		η_{sp}	% Recovery		η_{sp}	% Recovery	
		Sup	Pel		Sup	Pel		Sup	Pel
Yeast, no KCl	0	100	0	0	100	10	0	100	0
Yeast, 0.1 M KCl	0.22	13	90	0	44	60	0	37	73
Muscle, 0.1 M KCl	0.22	0	67	0.18	5	89	0.14	13	67

[a] Actin samples (0.2 mg/ml) in buffer D (0.1 mM $CaCl_2$) or buffer D plus 0.3 mM EGTA were incubated for 3 h at room temperature after additions of 0.1 M KCl or an equivalent volume of buffer D. Viscosity was measured at 26°C. Excess Ca^{2+} (0.6 mM) was added to a portion of the EGTA sample, and the effect on viscosity was measured.

[b] η_{sp}, Specific viscosity.

[c] Samples were centrifuged for 12 h at 105,000 × g at 20°C. Pellets were resuspended and dialyzed in buffer D. Protein recovered in the supernatant (Sup) and pellet (Pel) fractions is expressed as a percentage of the starting fraction. Long incubations at this temperature resulted in some loss of protein.

tin samples (0.2 mg/ml in buffer D or buffer D plus 0.3 mM EGTA) were incubated for 3 h at room temperature after the addition of 0.1 M KCl or an equivalent volume of buffer D. Viscosity was measured at 26°C. Excess Ca^{2+} (0.6 mM) was added to a portion of each sample that contained EGTA, and the effect on viscosity was measured. Samples were then centrifuged for 12 h at 105,000 × g at 20°C. Sedimented material was suspended in buffer D, and both pellet and supernatant fractions were dialyzed for 12 h at 4°C in buffer D. Protein recovered in each of the fractions was measured, and the results are summarized in Table 1. In the absence of added KCl, all of the yeast actin was recovered in the supernatant fraction. Low levels of Ca^{2+} had only a small effect on the sedimentation of muscle actin in 0.1 M KCl. When yeast actin was incubated in 0.1 M KCl plus 0.3 mM EGTA, the reduced viscosity (1.1 ml/mg) and the fraction recovered in the pellet (90%) were similar to those obtained with muscle actin. Although the addition of excess Ca^{2+} to this sample lowered the specific viscosity to zero, 60% of the protein sedimented under conditions that did not sediment G-actin. The same result was obtained with yeast G-actin incubated in buffer D plus 0.1 M KCl. G-actin or small aggregates could account for the protein that was not sedimented.

Electron microscopy. The results of the sedimentation analysis suggested that Ca^{2+} did not cause a complete conversion to G-actin. The possibility that Ca^{2+} produced some other form of actin was examined by electron microscopy. Yeast actin (0.2 mg/ml) formed filaments and filament bundles in buffer D plus 0.1 M KCl and 0.3 mM EGTA (Fig. 2a and b). The addition of 0.6 mM $CaCl_2$ to this sample resulted in the complete absence of filaments and the appearance of particles 15 to 50 nm in diameter (Fig. 2c and d). When yeast G-actin was incubated in buffer D plus 0.1 M KCl, large amorphous aggregates, but no filaments, were seen (Fig. 2e and f). At high magnification (Fig. 2i), this amorphous material appeared to be aggregates of the particles seen in the Ca^{2+}-treated F-actin sample. The addition of 0.3 mM EGTA to this sample resulted in the appearance of large numbers of filaments and filament bundles, whereas particulate material was no longer apparent (Fig. 2g and h). The large amount of particulate material seen in the presence of Ca^{2+} at low yeast actin concentration and its absence at higher actin concentrations suggest it is not an unrelated contaminant. Filaments and filament bundles, but no particulate material, were seen in buffer D plus 0.1 M KCl when the actin concentration was above the apparent C_∞ (Fig. 2j). Yeast actin (0.3 mg/ml) in buffer D plus 0.1 M KCl and 0.1 mM $MgCl_2$ formed only filaments and bundles (Fig. 2k). It should be noted that both yeast and muscle actins formed filament bundles under all polymerizing conditions tested. This might be an artifact of the sample preparation technique or could represent a functional property of the actin fractions.

Copolymerization of yeast and muscle actins. A number of proteins which cause a Ca^{2+}-dependent reduction in the viscosity of F-actin have been described previously (4, 8, 9, 17, 18). These proteins, isolated from a variety of cell types, cause a rapid response even at factor/actin molar ratios as low as 1:1,000. We have attempted to detect the presence of a similar factor in purified yeast actin samples by examining the polymerization of a mixture of yeast and muscle actins. In the experiment shown in Fig. 3 a constant amount (0.3 mg/ml) of yeast actin was incubated with various amounts of muscle actin in buffer D

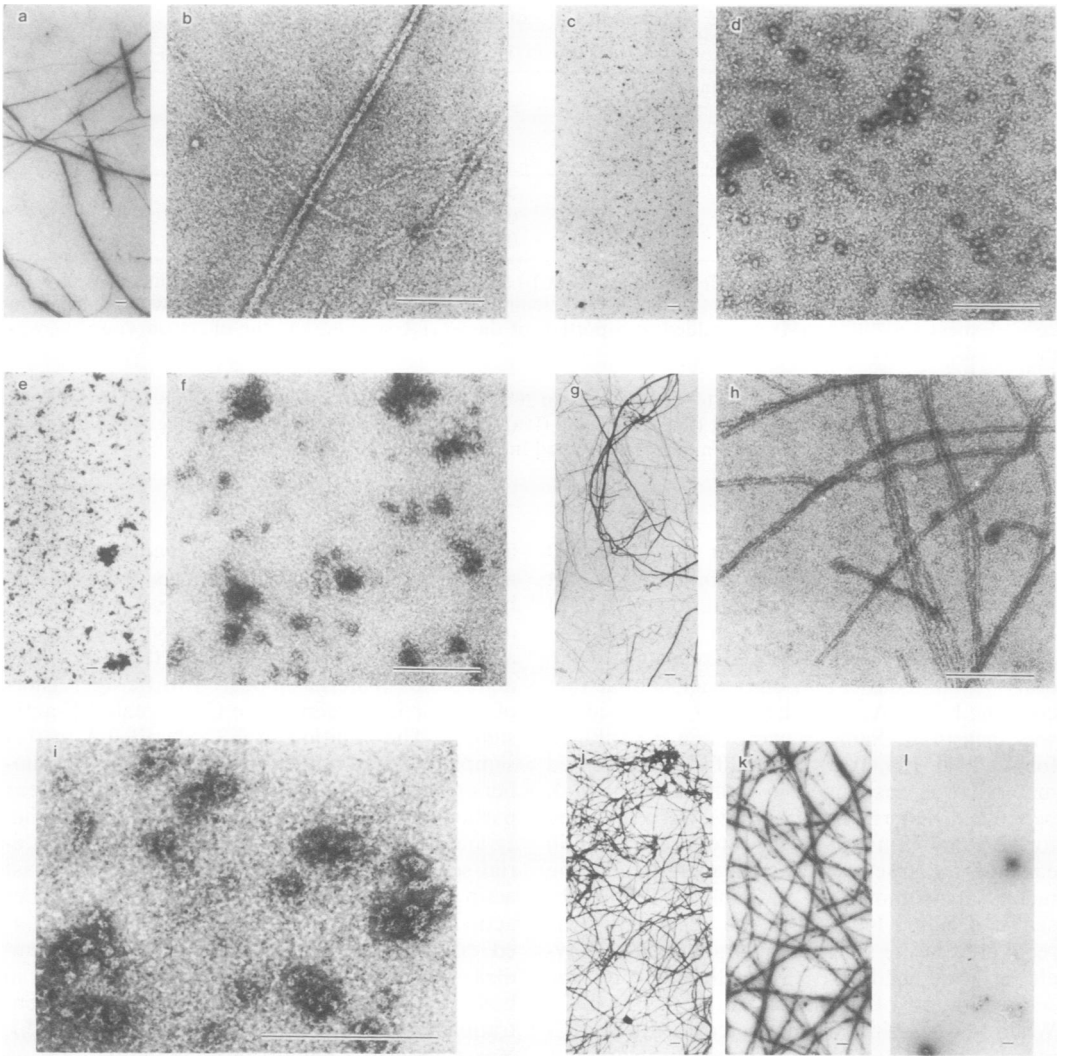

FIG. 2. Electron microscopic analysis of the effect of Ca^{2+} on polymerization of yeast actin. Samples were prepared for electron microscopy as described in the text. Bars, 200 nm. Panels: a and b, yeast actin (0.2 mg/ml) polymerized in buffer D plus 0.1 *M* KCl and 0.3 m*M* EGTA; c and d, excess Ca^{2+} (0.6 mM) was added to sample shown in a; e and f, yeast actin (0.2 mg/ml) polymerized in buffer D plus 0.1 M KCl; g and h, excess EGTA (0.3 mM) was added to sample shown in e; i, high magnification of aggregates in the sample shown in e; j, yeast actin (0.8 mg/ml) polymerized in buffer D plus 0.1 M KCl; k, yeast actin (0.3 mg/ml) polymerized in buffer D plus 0.1 M KCl and 0.1 mM $MgCl_2$; l, buffer D plus 0.1 M KCl.

plus 0.1 M KCl. In this experiment, the C_∞ for KCl-induced polymerization of muscle actin alone was 0.07 mg/ml in the presence or absence of Ca^{2+}. In the presence of yeast actin, the polymerization curve was biphasic. At low ratios of muscle to yeast actin, the slope was greater than that of muscle actin alone. Extrapolating this portion of the curve to zero viscosity gave a C_∞ of 0.37 mg/ml, which, when corrected for the yeast actin concentration, showed that the C_∞ for muscle actin was not affected by the presence of the yeast fraction. At higher muscle actin/yeast actin ratios, the curve was parallel to that of muscle actin alone, but offset by about 0.15 mg/ml. This suggests that only a portion of the yeast actin was capable of copolymerizing with muscle actin. Extrapolating this portion of the curve to zero viscosity gave an apparent C_∞ of 0.23 mg/ml. The addition of excess EGTA to these samples partially reversed the effect of Ca^{2+} in a manner similar to that seen with yeast actin alone (Fig. 1A): The apparent C_∞ was

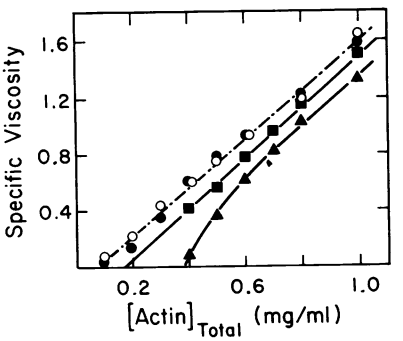

FIG. 3. Polymerization of a mixture of yeast and muscle actins. Samples containing muscle actin alone (●) or 0.3 mg of yeast actin per ml with various amounts of muscle actin (▲) in buffer D were incubated 3 h at room temperature after the addition of 0.1 M KCl. Viscosity was measured at 26°C. Excess EGTA (0.3 mM) was added, and samples were incubated for 1 h at room temperature. The resulting viscosity of the muscle actin (○) and yeast plus muscle actin (■) samples were measured at 26°C.

lowered to 0.17 mg/ml while the slope remained constant.

Fractionation of calcium-treated F-actin. In the presence of Ca^{2+} a portion of yeast actin did not copolymerize with muscle actin. This suggested that yeast actin may have two forms which differ in Ca^{2+} sensitivity. To test this possibility, G-actin produced by the addition of Ca^{2+} to F-actin was obtained by gel filtration, and the remaining polymerized or aggregated fraction was collected by sedimentation.

Yeast actin (1.05 mg/ml in buffer D) was polymerized for 3 h at room temperature after the addition of 0.1 M KCl and 0.3 mM EGTA. The reduced viscosity of this sample was lowered 40% by the addition of 0.6 mM Ca^{2+}. To obtain the fraction depolymerized by Ca^{2+}, a 2-ml sample was applied to a Sephadex G-150 column equilibrated in buffer D plus 0.1 M KCl at room temperature. Fractions (2 ml) were collected at a flow rate of 12 ml/h. The protein and actin profiles are shown in Fig. 4. The included peak of actin activity corresponded to the elution volume of muscle G-actin (determined in buffer D without KCl). Fractions 15 through 25 were pooled and contained 38% of the protein applied to the column. This corresponds to a C_∞ (equal to the equilibrium constant for addition of monomer to polymer [6]) of 0.4 mg/ml, similar to the value determined by viscometry. Approximately 1% of the protein eluted in the void volume. The remainder was probably trapped in the column, as the top 2 cm of the column resin, removed and dialyzed against buffer D, contained 2% of the protein. The pooled included fraction was concentrated to 0.4 mg/ml by dialysis against Ficoll type 400 powder and dialyzed against buffer D. To obtain a fraction which was not depolymerized by Ca^{2+}, the remainder of the Ca^{2+}-treated sample was centrifuged for 12 h at 105,000 × *g* at 20°C. The pellet, containing F-actin or actin aggregates but not G-actin, was suspended (0.45 mg/ml) and dialyzed in buffer D. The recovery of protein in this fraction was 41%.

The effect of Ca^{2+} on these fractions was examined by viscometry and sedimentation. The results are summarized in Table 2. The viscosity of both fractions was increased by the addition of 0.1 M KCl and 0.3 mM EGTA. The reduced viscosity of the G-150 included fraction was lower than that of the starting material, possibly due to inactivation of actin since the specific DNase inhibition activity was similarly reduced (data not shown). The addition of 0.6 mM Ca^{2+} lowered the reduced viscosity to nearly zero. This effect was most dramatic with the treated fractions because the actin concentration was at the Ca^{2+}-enhanced C_∞. A significant amount of the protein in both fractions continued to sediment under conditions which did not sediment G-actin, as was found for the unfractionated material.

DISCUSSION

The polymerization of yeast actin in KCl is sensitive to Ca^{2+}. Low levels of calcium cause

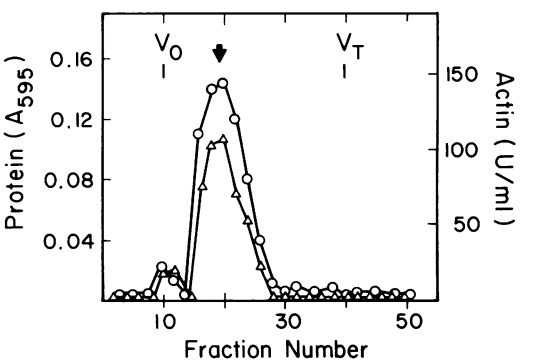

FIG. 4. Sephadex G-150 fractionation of Ca^{2+}-treated yeast F-actin. Yeast actin (1.05 mg/ml in buffer D) was polymerized for 3 h at room temperature with 0.1 M KCl and 0.3 mM EGTA. Calcium (0.6 mM) was added, and the sample was applied to a Sephadex G-150 column (1.5 by 29 cm) equilibrated in buffer D plus 0.1 M KCl at room temperature. Fractions (2 ml) were collected, and the protein content (△) (absorbancy at 595 nm in the dye-binding assay of Bradford [3]) and actin activity (○) were measured. The void volume (V_O) and included volume (V_T) were determined with bromcresol purple and blue dextran, respectively. The arrow marks the elution volume of muscle G-actin chromatographed on this column in buffer D.

TABLE 2. Calcium sensitivity of Ca^{2+}-treated F-actin fractions

Sample	KCl-EGTA[a]		With 0.6 mM $CaCl_2$[b]			
					% Recovery	
	η_{sp}	η_{red}	η_{sp}	η_{red}	Sup	Pel
Yeast actin	1.46	1.39	0.85	0.81	26	41
G150 included	0.27	0.68	0.01	0.03	13	44
105K pellet	0.54	1.20	0	0	13	51

[a] G-actin (G-150 included) and F-actin or aggregated actin fractions (105 × *g* pellet) of Ca^2-treated yeast F-actin were obtained as described in the text. Samples were incubated for 3 h at room temperature after the addition of 0.1 M KCl and 0.3 mM EGTA (KCl-EGTA). Values for reduced viscosity (η_{red}) are given in units of milliliters per milligram.

[b] Excess Ca^{2+} (0.6 mM) was added, and viscosity was again measured. Samples were centrifuged for 12 h at 105,000 × *g* at 20°C. Pellets were suspended and dialyzed in buffer D. Protein recovered in supernatant (Sup) and pellet (Pel) fractions is expressed as a fraction of the starting fraction. Long incubations at this temperature resulted in some loss of protein.

an eightfold increase in the concentration of actin required for polymerization (C_∞), as measured by high-shear viscometry. The effect is reversed rapidly by lowering the level of calcium with EGTA or by adding Mg^{2+} to concentrations below that required for Mg^{2+}-induced polymerization. The addition of calcium to preformed filaments also causes a reversible increase in the apparent C_∞. At actin concentrations above the apparent C_∞ the specific viscosity increase with increasing actin concentrations is nearly constant in the presence or absence of Ca^{2+}, indicating that the filament number and length distribution are similar. This is consistent with a uniform effect of Ca^{2+} on yeast actin.

Oosawa and co-workers (16) proposed that actin polymerization might occur only above a critical concentration analogous to a vapor pressure in gas condensation. In this model, F-actin is in equilibrium with a critical concentration of G-actin; below the critical concentration all actin is present as G-actin. By this definition, the Ca^{2+} effect on yeast actin is not strictly an increase in the C_∞. At actin concentrations below the Ca^{2+}-induced apparent C_∞ a significant fraction of the actin (~60%) sediments under conditions that do not sediment G-actin. Electron microscopy reveals that instead of filaments, yeast actin forms particles 15 to 50 nm in diameter. We have used the term apparent C_∞ to express the uncertainty introduced by the observation of Ca^{2+}-induced aggregates with low viscosity. Filaments formed in the presence of low levels of Mg^{2+} or at actin concentrations above the apparent C_∞ are indistinguishable from filaments formed in the absence of Ca^{2+}.

There are a number of proteins which cause a Ca^{2+}-dependent reduction in the viscosity of F-actin. These "severing" factors include villin isolated from intestinal epithelium (4), fragmin from *Physarum polycephalum* (8, 9), 95K protein from platelets (17), and macrophage gelsolin (18, 19). These proteins range in molecular weight from 42,000 to 95,000, have Ca^{2+} binding sites with micromolar dissociation constants, and bind to F-actin in the presence of Ca^{2+}. A decrease in the viscosity of F-actin is thought to be due to shortened filaments rather than to an increase in the amount of G-actin. The effect on filament length is proportional to the factor-actin stoichiometry and can be detected at ratios as low as 1:1,000. Gelsolin, for example, at a ratio of 1:98 causes a fourfold increase in the apparent C_∞ of muscle actin (1). The ability of these factors to provide enhanced nucleation sites for polymerization and to bind and cap one end may be related to their ability to shorten filaments.

The effect of Ca^{2+} on yeast actin is similar in some respects to the action of severing factors. In all cases, the Ca^{2+}-induced viscosity decrease is rapid, being complete within a few minutes. The Ca^{2+}-dependent effect of gelsolin on rabbit muscle actin does not result in a complete depolymerization to G-actin, as is also true of the Ca^{2+} effect on yeast actin. In preliminary experiments, yeast actin is sensitive to Ca^{2+} concentrations of less than 1 μM (data not shown), similar to the Ca^{2+} dependence of severing factors.

Several features distinguish the action of Ca^{2+} on yeast actin from the action of severing factors. First, low levels of Mg^{2+} reverse the effects of Ca^{2+} on yeast actin, but do not affect severing factor activity. Second, EGTA causes rapid and nearly complete recovery of yeast actin viscosity, whereas for gelsolin and fragmin the effect is reversed slowly (8, 19). Third, Ca^{2+} does not produce shortened yeast actin filaments as is seen with factor-treated muscle F-actin. Instead of short filaments, at yeast actin concentrations below the apparent C_∞, particles 15 to 50 nm in diameter are formed in response to Ca^{2+}. Fourth, at yeast actin concentrations above the

Ca^{2+}-induced apparent C_∞, the addition of Ca^{2+} to preformed filaments causes an increase in the amount of G-actin. When a Ca^{2+} depolymerized fraction is applied to a gel filtration column, 40% of the protein, corresponding to 0.4 mg/ml in the original sample, elutes in a peak with an elution volume equivalent to that of G-actin.

Although a Ca^{2+}-sensitive severing factor present as a contaminant in purified yeast actin may explain the behavior, we have been unable to obtain an actin fraction that does not respond to Ca^{2+}. A variety of alternative purification steps have been applied, including Sephadex gel filtration and DEAE-Sephadex chromatography in Ca^{2+}-free buffer and polymerization in KCl or Mg^{2+} followed by sedimentation. In no case is the Ca^{2+} effect diminished. The yeast actin purification procedure (including the Octyl-Sepharose step), when applied to muscle actin, did not change any characteristics of the muscle protein; i.e., the procedure itself did not cause Ca^{2+} sensitivity.

Yeast actin fractions have no severing factor activity as measured by their effects on muscle actin polymerization. The critical concentration curve for polymerization of a mixture of yeast and muscle actins in the presence of Ca^{2+} was complex (Fig. 3). The addition of muscle actin above its own C_∞ to yeast actin present at less than its C_∞ produced a viscosity increase larger than would be expected for muscle actin alone. This suggests that yeast actin, which would otherwise be unable to polymerize under these conditions, was capable of copolymerizing with muscle actin. Extrapolating the initial steep portion of this curve to zero viscosity indicates that the presence of the yeast actin fraction did not inhibit muscle actin polymerization. We also found that unlike extracts of *Physarum* (9), the soluble fraction of a yeast crude extract does not confer Ca^{2+} sensitivity upon muscle actin (data not shown). The addition of higher concentrations of muscle actin to a constant amount of yeast actin produced a viscosity less than that expected for complete polymerization of both actins. Extrapolating this portion of the curve to zero viscosity gives a C_∞ intermediate between that of either actin alone, suggesting that a fraction of the yeast actin (roughly half) did not copolymerize with muscle actin.

The limited ability of yeast actin to copolymerize with muscle actin in the presence of Ca^{2+} probably is not due to multiple species of yeast actin. *S. cerevisiae* has only one actin gene (5, 13), and the purified protein is a single isoelectric species (7). Furthermore, although Ca^{2+}-treated yeast actin can be separated into soluble and particulate fractions, both fractions retain Ca^{2+}-sensitive polymerization and sedimentability. This result also is inconsistent with the presence of a factor which binds to a fraction of the yeast actin.

All of these results are consistent with a direct effect of Ca^{2+} on yeast actin. Gordon et al. (6) have reported that 1 mM Ca^{2+} increases the C_∞ of both muscle and nonmuscle actins. For nonmuscle actins in particular, this effect is dependent on both high ionic strength (0.5 M KCl) and relatively high Ca^{2+} concentration (1 mM). Low levels of Ca^{2+} destabilize muscle F-actin in low-ionic-strength buffer (5 mM potassium phosphate [2]); this effect of Ca^{2+} on muscle actin is abolished by high ionic strength (50 mM potassium chloride). In contrast, the polymerization of yeast actin was sensitive to low levels of Ca^{2+} at KCl concentrations from 10 mM to 0.6 M. Nevertheless, the effect of Ca^{2+} on yeast actin is qualitatively similar to that observed with other actins.

The effect of Ca^{2+} on the polymerization of yeast actin may represent a mechanism for controlling the assembly state of actin. A rapid and dramatic change in actin assembly state in vitro occurs at actin and Ca^{2+} levels in the range of physiological concentrations. We estimate an intracellular actin concentration in yeast of about 0.5 mg/ml (7) which is close to the apparent C_∞ in the presence of Ca^{2+}. Although local concentrations may vary considerably, physiological Ca^{2+} concentrations are thought to be between 10^{-7} and 10^{-6} M (1). The assembly state of actin in vivo could be altered by changes in the local actin concentration and by regulation of the levels of free Ca^{2+} and Mg^{2+}. Mutant alleles of the yeast actin gene which influence polymerization or Ca^{2+} sensitivity (or both) will allow a cellular role to be ascribed to the properties of the purified protein.

ACKNOWLEDGMENTS

We thank Alice Taylor for expert technical assistance with electron microscopy, Robert B. Silver, James Spudich and members of his group, and W. Z. Cande for helpful discussions, and Peggy McCutchan for typing this manuscript.

This work was supported by grants from the National Institutes of Health (GM 26755 and ES 01896) and the National Science Foundation (PCM 80-19415).

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