Isolation and Characterization of a 30,000-dalton Calcium-sensitive Actin Cross-linking Protein from Dictyostelium discoideum

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A calcium-sensitive actin-binding protein having a subunit molecular mass of 30,000 daltons (30K protein) has been isolated from Dictyostelium discoideum. Structural, immunological, and functional analyses demonstrated that the 30K protein was distinct from other actin-binding proteins of D. discoideum. A native molecular mass of 31,700 daltons was determined by equilibrium sedimentation, indicating that the protein is monomeric. The Stokes radius was 30 Å. The frictional coefficient calculated from these measurements was 1.44, indicating an asymmetric shape. The 30K protein induced an increase in the viscosity of a solution of F-actin. Bundles of actin filaments were observed in negatively stained mixtures of actin and the 30K protein. Both the formation of filament bundles and the increases in viscosity of actin induced by the 30K protein were observed in the presence of $1 \times 10^{-8}$ M but not $2 \times 10^{-8}$ M calcium. Variation of the pH from 6.6 to 7.8 had no effect on the activity of the 30K protein. Calcium induced neither a large change in quaternary structure of the 30K protein nor a restriction of the lengths of actin filaments by the 30K protein. The apparent affinity of the 30K protein for actin was decreased in the presence of calcium. Reversible cross-linking of actin filaments by the 30K protein may contribute to regulation of the consistency and contractility of cytoplasm in D. discoideum.

Although the fundamental importance of changes in the structure of cytoplasm for cell movement has been suspected for over 100 years (reviewed by Allen, 1961), our knowledge of the structure of cytoplasm is rudimentary. Cytoplasm is a viscoelastic contractile substance capable of spatial and temporal changes in structure between solated, gelled, and contracting states (Taylor and Condeelis, 1979). The molecular architecture and function of these structural states of cytoplasm during cell movement remain to be elucidated.

Vegetative amoebae of the cellular slime mold Dictyostelium discoideum have been utilized extensively for cellular, ultrastructural, and biochemical investigations of cell movement and the structure of cytoplasm. Extracts of these cells form viscoelastic gels which contract on addition of calcium (Taylor et al., 1977; Condeelis and Taylor, 1977). Myosin-depleted fractions exhibit gelation, but not contraction. Gelation of the myosin-depleted fractions is inhibited in the presence of micromolar calcium or at a pH greater than 7.0 (Condeelis and Taylor, 1977; Hellewell and Taylor, 1979). Cytoplasmic structure may influence contractility, since conditions which induce solation of the myosin-depleted fractions initiate contraction in myosin-containing fractions (Condeelis and Taylor, 1977; Hellewell and Taylor, 1979; Taylor et al., 1979; Condeelis, 1981, a and b; Taylor and Fechheimer, 1982, a and b).

A number of proteins which may contribute to the structure and contractility of cytoplasm in D. discoideum amoebae have been identified. Actin and myosin have been purified from these cells and characterized extensively (Woolley, 1972; Uyemura et al., 1975; Kuczynski and Spudich, 1980). A 95,000-dalton protein which cross-links actin filaments at a free calcium ion concentration less than $10^{-7}$ M and a pH less than 7.0 comprises 1% of the protein in a soluble extract of these cells (Fechheimer et al., 1982; Condeelis and Vahey, 1982; Brier et al., 1983). D. discoideum amoebae also contain a calcium-insensitive actin-binding protein with a subunit molecular mass of 120,000 daltons (Condeelis et al., 1982), a 40,000-dalton protein which restricts the length of actin filaments in the presence of calcium (Brown et al., 1982; Yamamoto et al., 1982), calmodulin (Bazari and Clarke, 1981), and a partially purified calcium-insensitive actin-binding protein which modulates both the polymerization and depolymerization of actin filaments, possibly by interaction with the ends of actin filaments (Taylor et al., 1981). Such proteins are not unique to D. discoideum. Proteins which modulate the polymerization, length, and gelation of actin filaments have been isolated from a variety of cell types (Craig and Pollard, 1982; Korn, 1982).

The aim of this investigation was to characterize a calcium-sensitive actin-binding protein with a subunit molecular mass of 30,000 daltons that was originally identified in the contracted pellet fraction derived from soluble extracts of D. discoideum amoebae (Hellewell and Taylor, 1979). We found that the purified 30,000-dalton protein was monomeric and distinct from other contractile/cytoskeletal proteins present in these cells. Viscometry, electron microscopy, and sedimentation were used to show that addition of calcium inhibited gelation in mixtures of actin and the 30,000-dalton protein by reducing the apparent affinity of the 30,000-dalton protein for actin, thereby reducing the number of cross-links between the filaments. Preliminary accounts of this investigation have been presented (Taylor et al., 1981; Fechheimer and Taylor, 1983).

EXPERIMENTAL PROCEDURES

Materials—Pipes,$^2$ EGTA, aprotinin, phenylmethylsulfonyl fluoride, Tris, and EDTA were obtained from Sigma. Leupeptin and pepstatin were supplied by Vega Biochemicals, Tucson, AZ. ATP was purchased from Gibco, Gaithersburg, MD.

$^1$ M. Fechheimer, J. Brier, and D. L. Taylor, unpublished data.

$^2$ The abbreviations used are: Pipes, piperezine-N,N,N',N'-tetraacetic acid; EGTA, ethylene glycol bis-(β-aminoethyl ether)N,N,N',N'-tetraacetic acid; 30K protein, 30,000-dalton actin-binding protein; SDS-PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate.
purchased from Boehringer Mannheim. DEAE-Sephacel and Sephadex G-150 (superfine) were obtained from Pharmacia Fine Chemicals, Piscataway, New Jersey. Hydroxylapatite (fast flow) and diithio-riitol were supplied by Calbiochem Behring. Dithiobis(succinimidylpropionate) was obtained from Pierce Chemical Co. Fluorescamine was purchased from Hoffmann-LaRoche Inc.

Protein. DEAE-Sephadex, D. discoideum amoebic powder of rabbit skeletal muscle as previously described (Spudich and Watt, 1971), except that the actin was sedimented in the presence of 0.8 M KCl before dialysis. Actin was stored as previously described (Fowler and Taylor, 1980).

Filament was purified from chicken gizzard as described (Wang, 1977), using the modifications previously noted (Fechheimer et al., 1982).

The 30,000-dalton actin-binding protein was purified from D. discoideum amoebae (strain A3) which were grown and washed as previously described (Condeelis and Taylor, 1977). A contracted pellet fraction was prepared from these cells using a modification of the method of Helfenyi and Taylor (1979). The washed cells (50 g) were suspended in an equal volume of homogenization buffer (5 mM Pipes, 5 mM EGTA, 1 mM dithiothreitol, 4% sprotin, 0.2 mM phenylmethylsulfonyl fluoride, 10 μg/ml of leupeptin, 1 μg/ml of pepstatin, pH 6.8) and homogenized by explosive decompression of nitrogen after equilibration for 15 min at 250 p.s.i. in a Parr bomb (Parr Instrument Co., Moline, IL). The homogenate was sedimented at 110,000 × g for 40 min at 4°C, and the supernatant was collected. The extract was brought to 20 mM KCl, 1 mM MgCl₂, 1 mM ATP and then titrated to pH 6.8. The preparation was dialyzed for 15 h at 4°C against fresh equilibrating buffer to remove any contaminating actin and then sedimented at 27,000 × g for 15 min. The pellet was suspended in 100 ml of 10 mM Tris, 1 mM EDTA, 1 mM dithiothreitol, 0.1 mM ATP, pH 7.8, dispersed in a Dounce homogenizer, and dialyzed versus two changes of 2 liters of the same solution. After sedimentation at 27,000 × g for 15 min to remove actomyosin, the supernatant was dialyzed versus DEAE buffer (10 mM Tris, 0.5 mM EDTA, 0.5 mM dithiothreitol, 0.25 mM ATP, pH 8.5) and applied to a column (1.4 × 20 cm) of DEAE-Sephacel equilibrated with DEAE buffer. The 30K protein was not tightly bound to the column under these conditions and was eluted with 200 ml of a linear gradient of NaCl from 0 to 0.2 M in the equilibrating buffer. The column was eluted with 0.2 M NaH₂PO₄ in HAP buffer, pH 6.5.

Binding of 30K Protein to Actin—Proteins were mixed at room temperature and held for 2 h in 20 ml Pipes, 50 mM KCl, 1 mM MgCl₂, 1 mM ATP, 5 mM EGTA to the pH and calcium concentration indicated for each experiment. Actin was prepared for falling ball viscometry by dialysis versus 2 mM Pipes, 0.2 mM dithiothreitol, 0.2 mM ATP, 0.02 mM CaCl₂, 0.02% NaN₃, pH 7.0. After centrifugation at 200,000 g for 90 min, the G-actin was adjusted to 0.3% sodium deoxycholate and polymerized by addition of 5 mM MgCl₂ and 2 mM CaCl₂. The G-actin was mixed with other proteins, drawn into 100-μl glass capillaries, and held at 28°C for 1 h before determination of the apparent viscosity. Results presented are the average of triplicate determinations. The falling ball technique yields a semi-quantitative characterization of the consistency of these non-Newtonian solutions which is referred to in the text as apparent viscosity. Apparent viscosity was estimated by use of calibration curves as previously described (Fowler and Taylor, 1980).

Viscosity—Apparent viscosity was measured at low shear rates by the falling ball technique as previously described (MacLean-Fletcher and Pollard, 1980). Viscosity was measured in 20 mM Pipes, 50 mM KCl, 1.5 mM MgCl₂, 1 mM ATP, 5 mM EGTA at the pH and calcium concentration indicated for each experiment. Actin was prepared for falling ball viscometry by dialysis versus 2 mM Pipes, 0.2 mM dithiothreitol, 0.2 mM ATP, 0.02 mM CaCl₂, 0.02% NaN₃, pH 7.0. After centrifugation at 200,000 g for 90 min, the G-actin was adjusted to 0.3% sodium deoxycholate and polymerized by addition of 5 mM MgCl₂ and 2 mM CaCl₂. The G-actin was mixed with other proteins, drawn into 100-μl glass capillaries, and held at 28°C for 1 h before determination of the apparent viscosity. Results presented are the average of triplicate determinations. The falling ball technique yields a semi-quantitative characterization of the consistency of these non-Newtonian solutions which is referred to in the text as apparent viscosity. Apparent viscosity was estimated by use of calibration curves as previously described (Fowler and Taylor, 1980).

RESULTS Purification of the 30,000-dalton Protein—The 30,000-dalton protein was purified from the contracted pellet fraction of D. discoideum amoebae as described under "Experimental Procedures." Briefly, the amoebae were lysed by explosive decomposition of nitrogen in a Parr bomb, and a soluble extract was prepared by ultracentrifugation (Fig. 1, lanes b and c). The contracted pellet fraction was obtained from the extract by low speed sedimentation after addition of 25 mM KC, 1 mM MgCl₂, 1 mM ATP and titration of the pH to 7.5. Actin, myosin, and the 30,000- and 95,000-dalton actin-binding proteins were the most prominent polypeptides present in the contracted pellet fraction (Fig. 1, lane d). Myosin was removed by sedimentation after dialysis to low ionic strength (Fig. 1, lane e). The 95,000-dalton protein and actin were...
the high speed sedimentation equilibrium method as described under "Experimental Procedures." The logarithm of the concentration of protein was determined as a function of the square of the radial position in the centrifugal field after equilibrium had been attained (Fig. 2). The molecular weight calculated from the linear slope of the plot in Fig. 2 was 31,700 ± 400. Comparison of this value to the subunit molecular weight measured by gel electrophoresis indicated that the native protein was monomeric.

The Stokes radius of the 30K protein was determined by gel permeation chromatography in columns of Sephadex G-150. Elution of the 30K protein was detected by measurement of fluorescence after addition of fluorescamine to a portion of each fraction as described under "Experimental Procedures." The elution of the 30K protein from a column of Sephadex G-150 was not significantly affected by a change in the free calcium ion concentration from less than 1 x 10⁻⁶ M to 2 x 10⁻⁶ M (Fig. 3a). The Stokes radius of the 30K protein determined by calibration of the columns using proteins of known Stokes radii was 30 Å (Fig. 3b).

Interaction of the 30,000-dalton Protein with Actin—The interaction of the 30K protein with actin was characterized by viscometry, electron microscopy, and sedimentation. Addition of 20–30 µg/ml of 30K protein to a solution of 0.8 mg/ml of rabbit skeletal muscle F-actin induced a significant increase in the apparent viscosity (Fig. 4). The quantity of 30K protein required to increase the viscosity of 0.8 mg/ml of F-actin to greater than 500 centipoises varied from 10 to 40 µg/ml, depending on the preparations of 30K protein and actin.

The effects of free calcium ion concentration and pH on the apparent viscosities of mixtures of the 30K protein with actin were determined. The interaction of the 30K protein with actin was highly dependent on the free calcium ion concentration (Fig. 4). Elevation of the free calcium ion concentration to 10⁻⁷ M dramatically reduced the apparent viscosity of mixtures of the 30K protein with actin (Fig. 5). The apparent viscosity of mixtures of the 30K protein with actin was relatively insensitive to pH in the range from 6.6 to 7.8.

Binding of the 30K protein to actin filaments was assessed by co-sedimentation with actin filaments. Association of the 30K protein with actin was demonstrable in the presence of a low free calcium ion concentration (Fig. 6, lanes c and d) and was significantly reduced in the presence of micromolar calcium (Fig. 6, lanes e and f). Thus, an increase in the free calcium ion concentration reduced the apparent affinity of the 30K protein for actin.

Examination in the electron microscope of negatively stained mixtures of the 30K protein and actin revealed the presence of cross-linked bundles of actin filaments (Fig. 7). Single filaments and aggregates of two to three filaments were observed in solutions containing either actin filaments alone or mixtures of the 30K protein and actin in the presence of a free calcium ion concentration of 2 x 10⁻⁷ M. Variation of either the time allowed for bundle formation from 1 h to 3 days or the molar ratio of 30K protein to actin from 1:20 to 1:4 increased the size of the bundles and the extent of bundle formation (data not shown).

Viscometric and ultrastructural observations were performed in parallel on mixtures of the 30K protein and actin. Bundles of actin filaments were detected after 1 h in samples containing at least 30 µg/ml of 30K protein (one 30K protein monomer:20 actin monomers). The same concentration of 30K protein was required to induce a significant increase in the

**FIG. 1.** Analysis by SDS-PAGE of the polypeptide composition of fractions obtained during purification of the 30K protein. Lane a, molecular weight markers from top to bottom are: filamin (250,000), phosphorylase (94,000), bovine serum albumin (68,000), ovalbumin (40,000), and DNase (31,000); lane b, 28 µg of the homogenate; lane c, 26 µg of the solubilized extract; lane d, 13 µg of the contracted pellet fraction; lane e, 11 µg of protein soluble after dialysis of the contracted pellet fraction; lane f, 3 µg of 30K protein-containing fractions eluted from DEAE-Sephacel; lane g, 4 µg of purified 30K protein eluted from hydroxylapatite.

removed by chromatography in a column of DEAE-Sephacel (Fig. 1, lane f). The 30K protein was then purified by chromatography in a column of hydroxylapatite (Fig. 1, lane g). The yield of the 30K protein was 5–10 µg/g of washed cells.

**Structure of the 30,000-dalton Protein**—The amino acid composition of the 30K protein was distinct from that of actin, severin, and discoidin ¹ from *D. discoideum*. In addition, the composition of the 30K protein was distinct from that of a 36,000-dalton actin-binding protein from *Physarum polycephalum* and from that of human platelet tropomyosin (Table I). The partial specific volume of the 30K protein, calculated from its amino acid composition, was 0.73.

The structure of the 30K protein was investigated by gel electrophoresis, chemical cross-linking, sedimentation, and gel permeation chromatography. The apparent subunit molecular mass was 30,000 daltons as determined by SDS-PAGE (Fig. 1). Omission of the reducing agent β-mercaptoethanol from the sample did not alter the mobility of the 30K protein (data not shown). The mobility of the 30K protein was also unchanged after addition of the chemical cross-linking agent dithiobis(succinimidy1propionate) (data not shown). These results provided no indication that the native protein was composed of more than one 30,000-dalton polypeptide.

The native molecular weight was directly determined by

¹ Discoidin I was selected for comparison because it is present in the contracted pellet fraction of *D. discoideum* amoebae (M. Clarke, personal communication, Albert Einstein School of Medicine).
Dictyostelium 30,000-dalton Protein

TABLE I

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<th>Amino acid composition of the D. discoideum 30,000-dalton protein</th>
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ND, not determined.

* Data from Woolley, 1972.
* Data from Yamamoto et al., 1982.
* Data from Frazier et al., 1975.
* Data from Cohen and Cohen, 1972.
* Data from Ogihara and Tonomura, 1982.
* ND, not determined.
* Determined as cysteic acid after hydrolysis in the presence of dimethyl sulfoxide (Spencer and Wold, 1969).

The 30K protein (0.4 mg/ml) was sedimented for 21 h at 4 °C at 26,000 rpm in an AN-D rotor in the presence of 20 mM Pipes, 50 mM KCl, 1 mM EGTA, 1 mM EDTA, 1 mM dithiothreitol, 0.02% NaN3. Concentration at each radius was determined by analysis of Rayleigh interference fringes using a Nikon microcomparator.

Fig. 2. Analytical equilibrium sedimentation of the 30K protein. The 30K protein (0.4 mg/ml) was sedimented for 21 h at 4 °C at 26,000 rpm in an AN-D rotor in the presence of 20 mM Pipes, 50 mM KCl, 1 mM EGTA, 1 mM EDTA, 1 mM dithiothreitol, 0.02% NaN3. Concentration at each radius was determined by analysis of Rayleigh interference fringes using a Nikon microcomparator.

We also observed that the 30K protein did not significantly reduce the lengths of actin filaments in the presence of micromolar calcium. This issue was examined by measurement of the apparent viscosities of solutions containing either actin, chicken gizzard filamin, and the 30K protein or actin and filamin alone (Fig. 8). The 30K protein and filamin both increased the apparent viscosity of a solution of actin filaments when tested in the presence of a low free calcium ion concentration. In the presence of micromolar calcium, the apparent viscosities of mixtures of actin, filamin, and the 30K protein were identical to the apparent viscosities of mixtures of actin and filamin alone (Fig. 8). Since proteins such as gelsolin and villin reduce the apparent viscosity of mixtures of actin and filamin by restricting the lengths of actin filaments in the presence of micromolar calcium (Yin et al. 1980;...
been isolated from the contracted pellet derived from a soluble cell extract of the 30K actin-binding protein, and a few other poly-peptides. The presence of the 30K protein in the contracted pellet fraction (Taylor, 1979). The molecular mass of 31,700 daltons as determined by sedimentation, viscometry, and electron microscopy. Binding of the 30K protein to F-actin is directly demonstrated by sedimentation (Fig. 6). Increases in the viscosity of actin solutions are induced by addition of the 30K protein (Fig. 4), presumably due to cross-linking of actin filaments by the 30K protein. Formation of bundles of actin filaments in the presence of the 30K protein confirms that this protein cross-links actin (Fig. 7). Formation of complexes of actin and the 30K protein detectable by viscometry and electron microscopy is observed at molar ratios of 30K protein to actin greater than 1:20. The activity of the 30K protein is never observed in the presence of a free calcium ion concentration of $10^{-6}$ M by sedimentation, viscometry, or electron microscopy (Figs. 4-7). It is interesting to note that bundles of actin filaments are not observed in mixtures of actin and the D. discoideum 95,000-dalton calcium-sensitive actin-binding protein examined 1 h or 2 days after mixing at molar ratios up to 1:14.4.

Formation of bundles of actin filaments in mixtures of actin and the 30K protein is readily demonstrable as described above. However, these observations do not show that filament bundles are the only structures that form in these reconstituted mixtures, since randomly cross-linked filament networks are more difficult to detect in such preparations. Furthermore, our results do not allow a definitive statement concerning the structures which actin and the 30K protein might form in living cells.

We have examined three mechanisms that could potentially explain the inhibition of gelation in mixtures of actin and the 30K protein in the presence of micromolar calcium. First, the ability of the 30K protein to cross-link actin could depend on a self-association of the 30K protein that is inhibited by micromolar calcium. This hypothesis is not supported by the observation that a change in free calcium ion concentration has no detectable effect on the quaternary structure of the 30K protein (Fig. 3a). Moreover, the 30K protein is monomeric when examined in the presence of a low free calcium ion concentration in which its ability to cross-link actin is demonstrable.

Second, the 30K protein could inhibit gelation of actin in the presence of calcium by restricting the lengths of actin filaments, as proposed for gelsolin and villin (Yin et al., 1980; Nunnally et al., 1981). No evidence for filament cutting is

**DISCUSSION**

A 30,000-dalton calcium-sensitive actin-binding protein has been isolated from the contracted pellet derived from a soluble cell extract of *D. discoideum*. The contracted pellet fraction (Fig. 1, lane d) includes myosin, actin, the 95K actin-binding protein, the 30K actin-binding protein, and a few other polypeptides. The presence of the 30K protein in the contracted pellet, which exhibits calcium- and pH-regulated gelation, solvation, and contraction, suggests a role in the regulation of cytoplasmic structure and/or contractility (Hellewell and Taylor, 1979).

The 30K protein is structurally distinct from other cytoskeletal/contractile proteins of *D. discoideum* as assessed by amino acid analysis (Table I) and peptide mapping (Fechheimer et al., 1982). In addition, the results of immunodiffusion indicate that the 30K protein and the *D. discoideum* 95,000-dalton actin-binding protein do not share antigenic determinants (data not shown). The actin-binding sites of the 30K protein, as assessed by SDS-PAGE (Fig. 1) and a native gel filtration chromatography, is 30 kDa (Fig. 3b). These results indicate that the protein has an asymmetric shape. The unhydrated molecule has a frictional ratio of 1.44 and an axial ratio of 8, assuming a prolate ellipsoidal shape. If one assumes a hydration of 0.2 g of H$_2$O/g of protein, then the frictional coefficient and axial ratio of the hydrated prolate ellipsoid are 1.33 and 6.4, respectively. The radii of this prolate ellipsoid are 12 and 78 Å.

Interaction of the 30K protein with actin was studied by sedimentation, viscometry, and electron microscopy. Binding of the 30K protein to F-actin is directly demonstrated by Fig. 4 (left). The apparent viscosity of mixtures of the 30K protein with actin in the presence of a low or high free concentration of calcium. Apparent viscosity was determined by the falling ball technique in the presence of 20 mM Pipes, 50 mM KCl, 1.5 mM MgCl$_2$, 1 mM ATP, 5 mM EGTA, and calcium/ETGA ratios of 0.01 (○) and 0.9 (□), pH 7.4. The concentration of rabbit skeletal muscle actin was 0.8 mg/ml.

Fig. 5 (right). The effect of the free calcium ion concentration on the apparent viscosity of mixtures of the 30K protein with actin. Apparent viscosity was determined by the falling ball technique. Samples containing 0.8 mg/ml of rabbit skeletal muscle actin (□) or 0.8 mg/ml of actin plus 22 µg/ml of 30K protein (○) in the presence of 20 mM Pipes, 50 mM KCl, 1.5 mM MgCl$_2$, 1 mM ATP, 5 mM EGTA, pH 7.0, and the free calcium ion concentrations indicated. The two data points indicated EGTA had calcium/EGTA ratios of 0.01. cp, centipoise.
Fig. 6. Binding of the 30K protein to actin assessed by sedimentation and SDS-PAGE. The 30K protein (55 µg/ml) and/or rabbit skeletal muscle actin (2 mg/ml) were held for 2 h at room temperature in the presence of 20 mM Pipes, 50 mM KCl, 1 mM MgCl₂, 1 mM ATP, 5 mM EGTA, and either 0.25 or 4.5 mM CaCl₂. After sedimentation for 30 min at 23 p.s.i. in the airfuge, the presence of the 30K protein and actin in the supernatant and pellet fractions was determined by SDS-PAGE. Samples analyzed were: supernatant (lane c) and pellet (lane d) of actin at low calcium; supernatant (lane e) and pellet (lane f) of actin plus the 30K protein at low calcium; supernatant (lane g) and pellet (lane h) of the 30K protein at low calcium; supernatant (lane i) and pellet (lane j) of the 30K protein at high calcium. The positions of actin and the 30K protein are indicated.

Fig. 7. Cross-linking of actin filaments by the 30K protein observed by electron microscopy. Samples contained 0.8 mg/ml of rabbit skeletal muscle F-actin (A) or 0.8 mg/ml of actin plus 0.12 mg/ml of the 30K protein (B and C). Proteins were mixed in the presence of 20 mM Pipes, 50 mM KCl, 1.5 mM MgCl₂, 1 mM ATP, 5 mM EGTA, and calcium/EGTA ratios of less than 0.01 (A and C) or 0.9 (B), pH 7.0. After dilution with 10 volumes of buffer, the samples were applied to grids, negatively stained with 1% aqueous uranyl acetate, and examined in a Phillips 300 transmission electron microscope at an accelerating voltage of 60 kV. Magnification, × 56,000.

Fig. 8. The apparent viscosity of mixtures of actin with chicken gizzard filamin and the 30K protein in the presence of a low or high free concentration of calcium. Apparent viscosity was determined by the falling ball technique in the presence of 20 mM Pipes, 50 mM KCl, 1.5 mM MgCl₂, 1 mM ATP, 5 mM EGTA, and calcium/EGTA ratios of 0.01 (○ and △) or 0.9 (● and ▲), pH 6.8. Samples contained filamin plus 0.8 mg/ml of F-actin (△ and ▲) or filamin, 0.8 mg/ml of F-actin, and 10 µg/ml of the 30K protein (○ and ●).

molar free calcium ions does not support this hypothesis (Fig. 8).

Third, an increase in the free calcium ion concentration could inhibit gelation of actin by the 30K protein by reducing the apparent affinity of the 30K protein for actin. This hypothesis is supported by the observation that binding of the 30K protein to actin, assessed by co-sedimentation, is reduced in the presence of micromolar calcium (Fig. 6). Thus, the free calcium ion concentration regulates gelation in mixtures of actin and the 30K protein in vitro by controlling the number of cross-links between the filaments.

Changes in the free calcium ion concentration can modulate both the consistency and contractility of cytoplasmic extracts, and the movement of living cells (see Taylor and Condeelis, 1979; Hellewell and Taylor, 1979; Condeelis, 1981a and b; Taylor and Fechheimer, 1982). D. discoideum amoebae contain a number of calcium-sensitive cytoskeletal/contractile proteins including the 30K protein, a 95,000-dalton actin cross-linking protein (Fechheimer et al., 1982; Condeelis and Vahey, 1982; Brier et al., 1983), a 40,000-dalton protein that restricts the length of actin filaments in the presence of calcium (Brown et al., 1982; Yamamoto et al., 1982), and calmodulin (Bazari and Clarke, 1981). Investigation of the distribution and interactions of these proteins both in reconstituted systems and in living cells is required to elucidate the molecular structure of cytoplasm and the role of calcium in regulating cell movements.

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