Induction of either Contractile or Structural Actin-based Gels in Sea Urchin Egg Cytoplasmic Extract

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ABSTRACT

The gel formed by warming the 100,000 g supernate of isotonic extracts of sea urchin eggs to 40°C is made up of actin and two additional proteins of mol wt of 58,000 and 220,000. Actin and 58,000 form a characteristic structural unit which has now been identified in the microvilli of the urchin egg and in the filopods of urchin coelomocytes. However, egg extract gels did not contract as those from other cell types do, and the aim of these experiments was to determine the reason for this lack of contraction.

Although the extracts are dialyzed to a low ionic strength, myosin is present in insoluble form and makes up ~1% of the protein of the extract. It becomes insoluble in the presence of high ATP concentrations at 0°C, and the precipitate formed under these conditions consists almost entirely of myosin. This procedure provides a simple method of isolating relatively pure myosin without affecting other extract components and functions.

Contraction will follow gelation in these extracts if the temperature and time of incubation used to induce actin polymerization are reduced to minimize myosin inactivation. At the optimal ATP and KCl concentration for contraction, the contracted material has an additional 250,000 component and contains very little 58,000. The conditions found to provide maximum gel yields favor the formation of the actin-58,000-220,000 structural gel, while reduced temperature and increase in KCl concentration results in a contractile gel whose composition is similar to those reported from amoeboid cell types. Both the structural protein cores found in the egg microvilli and a gel contraction related to the amoeboid motion which is seen in later urchin embryonic development can thus be induced in vitro in the same extract.

Extracts of unfertilized eggs of the sea urchin Tripneustes gratilla made under isotonic conditions contain significant amounts of actin which remains in the unpolymerized form at 0°C. After dialysis to 10 mM PIPES buffer, actin polymerization can be induced in these extracts by the addition of 1 mM ATP and low KCl (10-20 mM). Polymerization is very slow (6-8 h) at 25°C, but is complete in 1 h at temperatures of 35°-40°C (15). The F-actin formed during warming combines with two additional proteins of the extract, with mol wt of 58,000 and 220,000, to form a gel network visible in the light microscope. This gel can be separated from the extract by centrifugation and is soluble in 0.6-1 M KCl. Reconstitution experiments were done by separating the gel components into an F-actin solution and a second solution containing the 58,000 and 220,000 proteins. When recombined, these components form gel identical in structure to that formed in warmed extracts. As might be expected, because the actin was already in the polymerized form and the gel not markedly temperature sensitive, this recombination can be carried out at both 25° and 0°C (16).

The three protein components of these gels have recently been isolated and purified (3). This allows the gelation process to be separated into two steps: the first is the combination of F-actin with the 58,000 protein to form rodlike aggregates visible in the light microscope, which were termed needles, and the addition of the 220,000 protein to these needles causes them to aggregate into a network similar in appearance to the gel formed in warmed extracts. The isolated 220,000 protein does not interact with actin in the absence of 58,000, indicating that it binds the needles via the 58,000 and not the F-actin and thus differs in function from other high molecular weight actin-binding proteins that have been described (13, 27).

In the electron microscope the gel has an unusual banding pattern with a repeat at ~110 Å (16). The separation and recombination of the gel components shows that this pattern is present in the actin + 58,000 needles and is caused by the
linkage of the F-actin filaments by 58,000 molecules, with the actin; 58,000 ratio measured at 4.6:1 (3). These structures have been analyzed in detail by optical diffraction and image reconstruction methods by DeRosier et al. (9), and a model in which the hexagonally packed F-actin units are cross-linked by the 58,000 protein has been devised. There are 41 actin monomers in the repeating unit, and the geometry allows the formation of nine bonds between the actin helices within this unit, spaced at 110 Å. If each band is formed by one 58,000 molecule, the ratio of this protein to actin will be 41/9 or 4.55.

These proteins that interact with actin in sea urchin egg extracts differ from those described in the gelation and contraction of amoeboid cell extracts (7, 21, 24, reviewed in reference 25) and their cellular role was unknown at the time they were first reported by the author, but the unique 110 Å banding pattern characteristic of the actin-58,000 combination has since been seen in two cellular organelles of the sea urchin. In the coelomocytes of the adult sea urchin, investigated by Edds (10, 11), the filopods formed by these cells in response to environmental stimuli were found to have a banding pattern similar to that of the actin-58,000 combination and these have recently been examined by optical diffraction methods (8). Antibodies to the 58,000 protein from the sea urchin egg (now named “fascin”) were used by Otto et al. (20) to demonstrate that a similar protein could be detected in the cytoplasm of the coelomocytes and was involved in the organization of F-actin filaments to the bundles which formed the core of the filopods. Burgess and Schroeder (5) observed a similar banding pattern in the microvilli formed after fertilization of the sea urchin egg, where a bundle of F-actin filaments also acts as a structural core for the microvilli and these have been analyzed by Spudich and Amos (23), who obtained evidence for a structure similar to that proposed by DeRosier et al. (9). The presence of 58,000 protein in egg microvilli has recently been demonstrated by immunofluorescence methods (4).

These results clearly demonstrate that the actin gel components first observed in vitro in sea urchin egg extracts play a structural role in both the coelomocyte filopod and the egg microvilli. Although an actomyosin contraction has been reported in an extract from another urchin species under different conditions (1), the actin-58,000–220,000 gels prepared in our experiments did not contract nor contain myosin as gels from amoeboid cells do. These observations, coupled with the presence of a unique 58,000 protein which causes the formation of highly organized actin aggregates, might suggest that this system is primarily structural and serves as a framework for such extended organelles as filopodia and microvilli. The object of the present investigation was to determine whether the lack of contraction was caused by the absence of myosin in the extracts as prepared or its inability to interact with actin under the conditions of the experiment. In the latter case, it might be possible to induce contraction by appropriate experimental modification and thus gain some insight into the cellular mechanisms controlling gelation and contraction.

MATERIALS AND METHODS

Extracts of the soluble proteins of unfertilized eggs of the Hawaiian sea urchin *Tripneustes gratilla* were prepared by methods similar to those described previously (15, 16). The washed and dejellied eggs are homogenized in a medium containing 0.9 M glycerol, 5 mM EGTA, and 0.1 M PIPES. The pH adjustment differs slightly from that of previous experiments, in that the pH is adjusted to 7.0 at 0°C throughout the procedure. The homogenate is centrifuged at 100,000 g for 1 h and the clear supernate is removed and dialyzed overnight at 0°-2°C to a concentration of 10 mM PIPES, 0.1 mM ATP and EGTA, pH 7.0. After dialysis, the extract is centrifuged at 25,000 g for 15 min and 1 mM EGTA and NaCl, are added. The total protein concentration of the extract is 10–12 mg/ml. The extract can be stored at 0°C for a few days, but gradually loses activity. At the suggestion of Dr. Joseph Bryan, we found that long-term storage could be accomplished by rapid freezing of the extract in liquid nitrogen, followed by storage at -80°C. Under these conditions the extract shows no loss of activity when stored for months.

K⁺-EDTA ATPase activity of the isolated myosin was measured in a medium containing 0.6 M KCl, 0.1 mM EDTA, 1 mM ATP, and 20 mM Tris-HCl at pH 8.0 and at pH 7.0 in the same medium with PIPES-NaOH substituted for Tris-HCl. The procedures used in ATPase measurement were similar to those utilized by Mabuchi (19) on starfish egg myosin, so as to obtain comparable values. Isolated myosin dissolved in 0.1 M KCl and frozen under the same conditions as extract showed no loss in ATPase activity after several months storage.

Sea urchin egg myosin dialyzed to a range of KCl concentrations from 0.5 to 0.35 M was negatively stained on Formvar-coated grids with 1% uranyl acetate and examined and photographed in a Philips 201 electron microscope operated at 60 kV.

SDS polyacrylamide slab gels were run at 7.5% using the procedures of Laemmli (18). The 0.75-mm slabs were stained for 1 h in 0.1% Coomassie brilliant blue in 95% ethanol:water:glacial acetic acid (5:5:1, by volume) and destained by diffusion in 10% acetic acid.

Protein measurements were made with the Hartree modification of the Lowry procedure (12), using a serum albumin standard.

ATP, GTP, ITF, EGTA, EDTA, and PIPES buffer were obtained from Sigma Chemical Co. (St. Louis, Mo.). Samples of isolated actin, 58,000 and 220,000 proteins were prepared as described previously (3). Samples of rabbit muscle actin and myosin and chicken gizzard filamin were the gift of Dr. Joseph Bryan, Department of Cell Biology, Baylor College of Medicine, Houston, Texas.

RESULTS

Presence and Separation of Myosin

Actin polymerization followed by gelation and myosin-based contraction has been reported in a variety of cell extracts, for example, *Acanthamoeba* (21), *Dictyostellium* (7), *Xenopus* (6), and rabbit macrophages (24). Although myosin has been isolated and characterized from echinoderm eggs (19), gelation was not followed by contraction in our previous experiments on urchin egg extracts (15, 16). This could be because of the absence of myosin from the final extract, as extraction is carried out under relatively low ionic strength conditions and the homogenate centrifuged at 100,000 g; the high-speed supernate is then dialyzed to 10 mM PIPES overnight and centrifuged at 25,000 g. The solubility properties of echinoderm egg myosin (19) indicate that it should be insoluble and lost from the extract during centrifugation.

However, myosin is present in a soluble form in the final extract and was first detected by its spontaneous precipitation during extended storage at 0°C. Dialyzed extracts held at 0°C retain their ability to gel for several days, but insoluble material slowly appears in the solution. The removal of this material by centrifugation did not affect gelation, and SDS polyacrylamide gel electrophoresis showed it to contain a polypeptide of the same mobility as rabbit muscle myosin heavy chain. This precipitation can be accelerated by the addition of high concentrations of ATP to dialyzed extract at 0°C. Precipitation begins at ~5 mM ATP and occurs more rapidly with increasing ATP concentrations to an upper limit of 10 mM; we routinely use 8 mM. The appearance of insoluble material begins a few minutes after ATP addition and the process is complete in 2–3 h at 0°C. The material is in the form of relatively uniform granules of sufficient size that they can be collected by centrifugation at 5,000 g for 10 min. The material is washed free of extract by suspending in 10 mM PIPES and recentrifuging at 25,000 g. When the resulting pellet is run on SDS polyacrylamide gel electrophoresis, it consists almost entirely of an ~200,000 mol wt polypeptide with the same mobility as rabbit myosin heavy chain (Fig. 1).
This protein forms typical myosin thick filaments in slightly higher KCl concentrations than reported for starfish egg myosin which had been isolated by ammonium sulfate fractionation and gel filtration (19). The salt concentration of a solution of sea urchin egg myosin precipitated from extract by 8 mM ATP and dissolved in 1 M KCl was slowly reduced by dialysis over several days and samples were removed for examination in the electron microscope. Thick filaments are present in 0.45 M KCl (Fig. 2), and at lower salt concentrations they exist in large aggregates. The length of the central bare zone of these filaments is ~0.15 nm and the overall length approximately twice this. The K+EDTA ATPase activity of this myosin (controls, Table I) is also similar to that reported for starfish egg myosin (19). The specific activity of sea urchin egg myosin at pH 8.0 is virtually identical to that of starfish egg myosin under the same conditions and both have a sharp decrease in activity as the pH is reduced below this optimum.

The yield of myosin in this procedure is of the order of 0.10–0.15 mg/ml extract, or ~1% of the extract protein. Although present in the soluble form in the extract, once precipitated by high ATP the myosin behaves conventionally and dissolves only in high salt solution and reprecipitates on the reduction of ionic strength. Reduction of ATP concentration by dialysis of the extract does not resolubilize the myosin. Precipitation of myosin from extracts is not specific for ATP and occurs with similar rate and yield with GTP and ITP.

**Temperature and Contraction**

If myosin is present in dialyzed urchin egg extracts, why is the initiation of actin polymerization by warming these extracts followed by gelation but not by contraction? The results presented above might suggest a requirement for a high ATP concentration to release the myosin from some bound or inhibited form, but an increase in ATP concentration to 8 mM in the procedure previously used (15) for gelation does not result in contraction. Changes in the free calcium concentration at the micromolar level also does not cause contraction in sea urchin egg extracts, although it has significant effects in other cytoplasmic contractile systems (see reference 25 for review). As in previous experiments (16) on these extracts, Ca/EGTA ratios above 0.8 prevent gelation, and ratios below this have no discernible effect. This effect may be mediated through a calcium-dependent regulatory protein as described in macrophages (28), because purified urchin gel components are not calcium sensitive (3).

The maximum yield of gel was obtained by incubating extracts containing millimolar ATP and low (10–20 mM) KCl at 40°C for 1 h (15). Lack of contraction under these conditions could be caused by the inactivation of myosin at this elevated temperature, as myxomycete myosin is completely denatured by incubation at 55°C for 15 min (14). Measurement of sea urchin egg myosin ATPase confirmed that incubation at 40°C for 1 h caused myosin inactivation (Table I). A temperature cycle was sought which would reduce the time for actin polymerization and gelation below the 6–8 h required at 25°C but allow the retention of myosin activity. The successful procedure devised involves a reduction in both the temperature and time.

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**Figure 1** SDS polyacrylamide gel electrophoresis. (A) Rabbit muscle myosin. (B) Myosin precipitated from urchin egg extract by 8 mM ATP, washed in 10 mM PIPES. (C) A + B.

**Figure 2** Thick filaments formed by sea urchin egg myosin on dialysis to 0.45 M KCl. Bar, 0.1 μm × 82,000.

**Table I**

| Treatment   | pH 8.0 | pH 7.0 |
|-------------|--------|--------|
| Control     | 314    | 38     |
| 40°C, 1 h   | 2      | 2      |
| 35°C, 10 min| 238    | 31     |

**Table 1** K+EDTA ATPase Activity of Urchin Egg Myosin

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of exposure. Warming extract containing 8 mM ATP and 25–50 mM KCl to 35°C for 10 min is sufficient to initiate some actin polymerization and also causes the appearance of myosin in insoluble form. On return to 25°C, slow gelation continues, contraction begins in ~1 h, and the gel is reduced to a small volume within 2 h. The optimum temperature range is 35° ± 2°C and an exposure time of 10–15 min results in a minimum contraction time. Myosin ATPase measurements showed that 75% of the activity was retained under these conditions (Table I).

With the use of this time-temperature cycle, the effect of ionic conditions on contraction can be determined. The pH of 6.8 used in previous gelation experiments lies near the lower limit for successful contraction, which slows at pH 6.7 and often fails at 6.6; the extracts used in these experiments were made at pH 7.0 and the final pH after dialysis was 6.9–7.0. Increase in pH to 7.3–7.5 does not induce actin polymerization in this extract as it has been reported to do in isolated urchin egg cortex (2). Contraction does not occur below 10 mM KCl and the optimum range of KCl concentration in terms of rate of contraction and protein yield is 25–50 mM, similar to the optimum reported in other cytoplasmic contractile systems (7, 21).

**Magnesium Addition**

Contraction of *Acanthamoeba* (21) and Dictyostelium (7) extracts was reported to be accelerated by the addition of 1 mM MgCl₂ in the presence of 8 mM ATP and 20–30 mM KCl. Added magnesium also reduces the time required for gel contraction in urchin egg extracts containing 8 mM ATP and 25 mM KCl, but the magnesium concentration cannot exceed that of ATP or gelation is blocked, as reported previously (15). If 4–6 mM MgCl₂ is added to an extract containing 8 mM ATP and 25 mM KCl, warmed to 35°C for 10 min, and returned to 25°C, contraction occurs more rapidly than in the absence of magnesium. Myosin becomes insoluble during warming and myosin granules are seen attached to the gel filaments formed (Fig. 3). The gel is visible in the solution as a slight turbidity and it can be seen to pull away from the walls of the tube within 10–15 min at 25°C. Contraction proceeds rapidly, the gel in a 1-ml sample reaching a volume of only a few microliters, or <1% of its original volume, in 20–30 min at 25°C.

The yield of contracted material doubles as the magnesium concentration is increased from 0 to 6 mM in the presence of 8 mM ATP. The major components (Fig. 4) of the contracted material in 8 mM ATP, 0 MgCl₂ are actin and myosin, with a smaller amount of unidentified polypeptide at 110,000. As the magnesium concentration is raised, two additional high molecular weight components at ~220,000 and 250,000 appear and become significant components. The 58,000 protein which is a major component of egg extract actin gels at 40°C (15), is not present in the contracted material under high ATP conditions.

**ATP and KCl Effects on Composition**

If the effect of added magnesium on contraction rate and yield is caused by the reduction of "free" ATP (that is, the ATP concentration in excess of magnesium concentration), then the
high ATP levels necessary for myosin precipitation may not be prerequisite for contraction. The minimum ATP concentration for contraction was determined to be 2 mM, and extracts containing 2 mM ATP instead of 8 mM ATP + 6 mM MgCl₂ warmed to 35°C for 10 min form a gel without visible myosin inclusions and begin contracting after 10–15 min at 25°C. At the same KCl concentrations, contractions are somewhat slower and final volumes larger than in the case of the high ATP and magnesium samples. The optimum range of KCl concentration for contraction is 25–50 mM and increased KCl, to an upper limit of 150 mM, increases the rate but decreases yield. Lower KCl concentrations lead to slower contractions with larger final pellets, with contraction ceasing and only gelation occurring below ~10 mM KCl.

At 25 mM KCl and 2 mM ATP, the composition of the contracted material (Fig. 5) is similar to that obtained under high ATP-Mg conditions, that is, actin, myosin, the 220,000 and 250,000 proteins, but some 58,000 protein is also present. The 250,000 polypeptide in these preparations has a mobility similar to a sample of the high molecular weight actin-binding protein filamin, which was isolated from vertebrate material (26). Increased KCl causes a decrease first of the 58,000 protein, and at the upper limit of 150 mM, increases the rate but decreases yield. Lower KCl concentrations lead to slower contractions with larger final pellets, with contraction ceasing and only gelation occurring below ~10 mM KCl.

Relation of Gelation and Contraction

The relation between gelation and contraction can be investigated using extract from which the myosin has been removed by the addition of 8 mM ATP. The precipitated myosin is collected by centrifugation and dissolved in 1 M KCl and the myosin-free extract then dialyzed to 10 mM PIPES buffer and 1 mM EGTA added after dialysis. The separated myosin is dissolved in a volume of 1 M KCl such that its addition to the extract returns the same amount of myosin removed and also adds the 25 mM KCl needed for gelation and contraction. When myosin dissolved in 1 M KCl is returned to the extract, 2 mM ATP is added and the preparation warmed to 35°C for 10 min, gelation and contraction occur as in control experiments and the contracted material has the same composition as untreated extract (Fig. 6). Virtually all of the myosin added to the extract is found in the contraction pellet but this is in excess of that required to contract the gel formed under these
conditions. If graded amounts of myosin are added to myosin-free extract before warming, contraction occurs with as little as 25% of the myosin present in control extract and contraction fails only when myosin is reduced to 10% of control values.

The separation and readdition of myosin can also be used to demonstrate directly its temperature inactivation. Samples of isolated myosin are incubated at 40°C for 1 h or at 35°C for 10 min before readdition to myosin-free extract and warming to 35°C for 10 min. Extract containing myosin previously warmed to 40°C gels but does not contract, while extract containing myosin previously warmed to 35°C contracts successfully. This is in agreement with the effect of these temperatures on ATPase activity (Table I), as the activity remaining after 35°C treatment is more than sufficient for contraction.

The gel obtained from myosin-free extract using the short 35°C incubation procedure devised for contraction has a different composition than that prepared in previous experiments by warming to 40°C for 1 h. The effects of incubation temperature and incubation time can be separately investigated by lengthening the time of incubation at 35°C from 10 min to 1 h, which causes a small increase in the 58,000 and 220,000 and decrease of the 250,000 (Fig. 6). Raising the incubation temperature to 40°C for 1 h markedly changes the composition, the 250,000 protein virtually disappears from the gel, while the 58,000 becomes a major component (Fig. 6). Actin, the 58,000 protein, and the 220,000 protein, in this proportion, made up the 40°C gels prepared and characterized from sea urchin egg extracts in previous experiments (15).

**DISCUSSION**

**Egg Myosin and Contraction**

Contrary to the reported solubility behavior of myosin isolated from echinoderm eggs (19), the extract of sea urchin eggs used in these experiments contains myosin in a form which remains soluble in low ionic strength solution. Myosin makes up ~1% of the total protein of the extract, considerably lower than the amount of actin, but this amount is in excess of that required to cause gel contraction. The cause of the low salt solubility is unknown, but the effect of high ATP concentrations suggests that an ATP induced dissociation of some component bound to the myosin might be involved. This ATP effect is irreversible, for once precipitated, the myosin displays conventional solubility behavior and the removal of ATP from the extract by dialysis does not restore the low salt solubility.

Whatever its mechanism, the solubility change induced by ATP provides a simple method for the separation of myosin from the extract. The material precipitated by ATP addition to the extract at 0°C consists almost entirely of myosin as characterized by electrophoretic mobility, ATPase activity, and thick filament formation. This procedure also allows the preparation of relatively pure myosin without damage to the other components of the extract—removal of the ATP by dialysis after myosin isolation results in an extract still capable of gelation. Readdition of myosin to such extracts restores contractility and makes possible the demonstration that the failure of contraction in earlier experiments on urchin egg extracts was caused by the inactivation of myosin at the temperature of 40°C used to initiate actin polymerization.

The appearance of myosin in insoluble form during the 10-min incubation at 35°C under high ATP conditions suggested that the ATP-induced change in myosin solubility might be a prerequisite for contraction. However, at the minimum ATP concentration for contraction of 2 mM, granular myosin is not seen attached to the filaments of the contracting gel, but electrophoresis shows that myosin is present in the contracted material. Myosin seems equally effective in contraction when present in visible, aggregated form or when dispersed in the gel in a form not apparent in the light microscope, but a large myosin excess is present and only a small fraction may be functioning in contraction in either case. Although speculations have been made about the interaction of myosin with actin gels in cell motility (discussed in reference 25), information at the ultrastructural level is still too limited to reach any conclusions about mechanism.

The properties of both actin and myosin are modified in egg extracts. Purified actin from *Tripneustes gratilla* eggs polymerizes rapidly at 25°C (16), while actin in egg extracts polymerizes very slowly at this temperature and extracts must be warmed to 35°-40°C to bring about rapid gelation. The temperature required to accelerate polymerization is 10°C above the normal environmental temperature of 25°C of these eggs, and the temperature cycle appropriate for this tropical species may not be effective for others (11). The present experiments also show that the myosin is present in extracts in soluble form under much lower salt concentration than is possible after its purification. This unusual behavior of both actin and myosin may be related to cellular control mechanisms. Cellular actin must be inhibited in some way from polymerizing throughout the cytoplasm at the normal physiological temperature of 25°C and through some unknown mechanism the inhibition is overcome at locations and times that are under cellular control. Likewise, myosin may be present in the cytoplasm in dispersed form and thick filaments formed only in localized areas when required. The rapidity with which contractile material appears and disappears in such functional areas as the cleavage furrow (22) indicates the level of control that must be involved.

**Relation of Composition and Behavior to Experimental Conditions**

Removal of the myosin results in an extract capable of gelation but not contraction. Except for the absence of myosin, the composition of the contracted material and the gel formed under the same experimental conditions are similar, indicating that myosin brings about contraction of the gel but does not have significant effects on the gelation process. It is therefore possible to consider the effects of experimental conditions on gelation without regard to the presence or absence of myosin.

Reduction in the incubation temperature from 40° to 35°C to prevent myosin inactivation also causes the appearance of a new 250,000 protein component in both the contracted material and in the gels. This component travels with the same mobility as a sample of vertebrate filamin and remains bound, presumably to the actin, in washed gels. A high molecular weight actin-binding protein of this type was first isolated from vertebrate macrophages (13) and a polypeptide of similar molecular weight has been seen in *Acanthamoeba* extract contraction (21) and also in *Dictyostelium* extracts (7). The evidence indicates that its absence from urchin gels prepared at 40°C is a direct result of the higher temperature, so a reduction in incubation temperature to 35°C causes the gelation behavior of urchin extract to more closely resemble that of extracts of other cell types in which actin polymerization takes place at 25°C.

Warming an extract containing 8 mM ATP, with no added
magnesium, results in contracted material of very simple composition. The myosin becomes insoluble under these conditions and this granular myosin associates with the actin polymerized at 35°C to form what is essentially an actomyosin pellet (Fig. 4B). As magnesium is increased from 0 to 6 mM (Fig. 4B-E), the 220,000 and 250,000 polypeptides become significant components of the contracted material. However, the appearance of these high molecular weight components is not a direct result of the added magnesium, but is caused by a reduction in the effective ATP concentration, as can be seen by comparing Fig. 4 E (8 mM ATP, 6 mM Mg^{2+}) with Fig. 5 C (2 mM ATP). Both contain similar 220,000 and 250,000 components and this is brought about equally as well by reduction in ATP concentration as by the addition of magnesium. Thus magnesium appears to have no obligatory role in the contraction process, although it does increase the rate.

The amount of 58,000 protein (fascin) in the contracted material is a function of the ATP and KCl concentrations. Contraction carried out under high ATP conditions, with or without added magnesium, results in contracted material with no detectable 58,000 present in the electrophoresis patterns. If ATP is reduced to 2 mM, the amount of fascin present in the contracted gel depends on the KCl concentration and the major difference between the contracted material formed in 25 mM KCl and the noncontractile gel formed in 10 mM KCl is an increase in the 58,000. This fact, coupled with the observation that gels with a higher proportion of fascin are more rigid, supports the hypothesis that the fascin-actin combination functions as a structural unit and as it increases in relative amount it leads to a rigid and noncontractile gel. The 220,000 protein is retained as a major gel component under conditions in which it is much in excess of the 58,000 present. In experiments with purified components (3), the 220,000 appeared to bind to the 58,000 and not to the actin and its binding site and role in the contracted material is unknown.

Thus the higher temperature used in previous urchin egg gelation experiments, coupled with the low ATP and KCl concentrations, resulted in the absence of myosin and the 250,000 protein from the gels and caused the formation of a rigid structural gel based on the interaction of F-actin and the 58,000 protein to form regularly cross-banded linear aggregates. These aggregates later proved to be similar in structure and composition to the protein cores of such extended cell organelles as the egg microvilli and the celloidinocyte filopods. Relatively minor changes in conditions will induce contractile behavior instead of the formation of these structural units in urchin egg extracts. Increase in the ATP concentration from 1 to 2 mM and in the KCl concentration from 10 to 25 mM, which reduce the 58,000 protein in the gel, coupled with a decrease in temperature and time of incubation to avoid myosin inactivation, results in the myosin-based contraction of an actin gel containing little or no fascin but with a 250,000 component characteristic of contracted material from several amoeboid cell types. The modification of cytoplasmic actin gels for rigidity or contractility during cellular activity thus involves different actin-associated proteins. While the cellular control mechanisms remain unknown, it is now possible to induce both the structural unit of the protein cores of the egg microvilli and a gel contraction related to the amoeboid motion of later urchin embryonic development in vitro in the same egg extract.

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