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THE CONTRACTILE PROTEINS OF DICTYOSTELIUM DISCOIDEUM

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We have purified actin and myosin-like proteins from amoebae of Dictyostelium discoideum. These proteins are very similar in their physical and enzymatic properties to muscle actin and myosin. Most importantly, they form thin and thick filaments, respectively, and Dictyostelium actin activates Dictyostelium myosin ATPase activity. Actin from these amoebae appears to be identical in size to muscle actin. The Dictyostelium myosin consists of two heavy chains of about 210,000 daltons and two classes of light chains, about 18,000 and 16,000 daltons. The heavy chains are slightly larger than those of muscle myosin. Biochemical and structural studies of membrane association of the contractile complex suggests that some of the amoeba actin is membrane-bound and acts as an attachment point for myosin and other actin filaments.

INTRODUCTION

The mechanism of force transduction in striated muscle has been extensively studied, and the major components of the muscle system have been well characterized. Contraction is produced by the interaction of actin and myosin filaments in a highly ordered structure called the sarcomere (Fig. 1).

Actin is a globular protein (42,000 daltons) which polymerizes to form 2-start helical filaments, 70 Å in diameter. These filaments have polarity, as has been demonstrated (1) by their interaction with myosin fragments, HMM* or S1, which decorate actin filaments to produce an arrowhead pattern (for example, see Fig. 2). In the sarcomere, actin filaments are attached to structures called Z-lines; when these filaments are decorated with HMM, all the arrowheads point away from the Z-lines (1).

Myosin is a high molecular weight fibrillar protein which has a long helical "tail"

*Abbreviations used are: DTT, dithiothreitol; SDS, sodium lauryl sulfate; HMM and S1, proteolytic fragments of myosin from rabbit striated muscle.

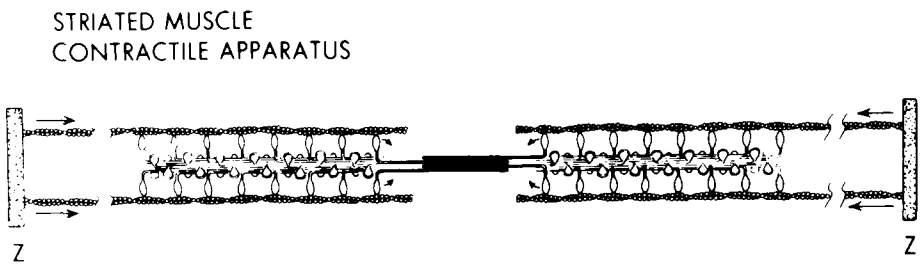

Fig. 1. Arrangement of filaments in a sarcomere of striated muscle. The polarity of the actin filaments is indicated by arrows which point in the same direction as the "arrowheads" formed by decoration of the actin with HMM. Note that actin filaments always point away from the Z-line, and that the myosin molecules which interact with an actin filament have a head-to-tail polarity that is the same as the polarity of that actin filament.

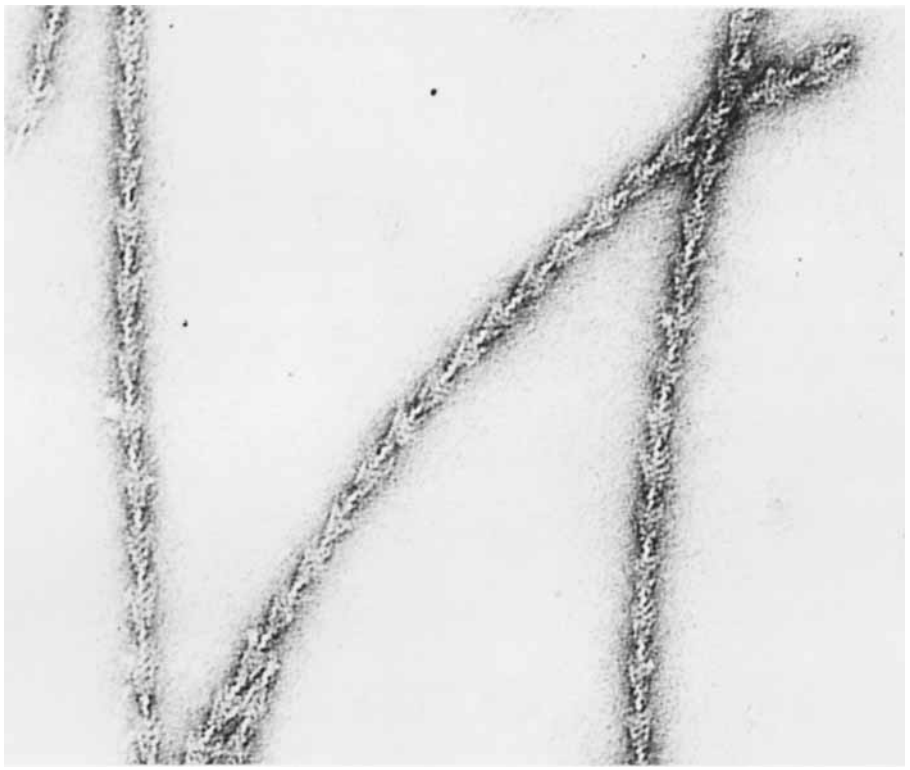

Fig. 2. Actin filaments decorated with S1. Filaments of muscle actin were decorated with S1 using the procedures described by Moore et al. (15). Actin was applied to a grid, which was then rinsed with 5 drops of 0.6 M KCl. An S1 preparation (0.5 mg/ml in 0.5 M KCl) was applied to the grid, and after 30 sec was rinsed away with a solution containing 0.2 mg/ml cytochrome c and 0.1% amyl alcohol. Uranyl acetate (1%) was then applied. Magnification 220,000 X.

and a globular "head." It consists of two heavy chains (200,000 daltons each) which make up the tail and part of the head region, and of three sizes of light chains (16,000–25,000 daltons), which are located in the globular heads. The head portion of the molecule contains ATPase and actin-binding activities. Under physiological conditions the ATPase activity of myosin is greatly stimulated by actin. Myosin tails interact with one another in solution to yield a bipolar filament with heads clustered at either end (2).

In the sarcomere each end of a bipolar myosin filament associates with actin filaments of the appropriate polarity. Hydrolysis of ATP by the myosin heads provides the energy to slide the two types of filaments past one another, presumably by movement of the myosin heads while attached to the actin.

It is commonly assumed that some similar mechanism is utilized in producing various types of nonmuscle movements such as cell division, single cell migration, endocytosis, and exocytosis. Proteins resembling muscle actin and myosin have been identified in many types of nonmuscle cells, not only from higher organisms, but also from the unicellular amoebae of Acanthamoeba and of Dictyostelium and from plasmodia of Physarum [for review, see (3)]. In general, these proteins closely resemble their muscle counterparts; the only apparent exception is a unique Acanthamoeba protein with myosin-like activity (4). However, ordered structures resembling sarcomeres have not been observed in nonmuscle cells; consequently the organizational basis by which movement is produced is not readily understood.

We have begun our study of the role of actomyosin-like proteins in nonmuscle cells by purifying and characterizing myosin and actin from amoebae of the cellular slime mold Dictyostelium discoideum. Woolley showed that this organism possesses an actomyosin-like complex (5) and described some of the properties of the isolated actin (6). We have purified the Dictyostelium myosin and actin, and examined their interaction with one another and with other cell components. This report summarizes these studies, which appear in more detailed form elsewhere (7, 8).

METHODS

Purification Procedures

Dictyostelium discoideum amoebae (strain A3, a gift of Dr. W. F. Loomis, Jr.) were grown in HL5 axenic medium (9) at 23° and were harvested in late exponential phase of growth.

For purification of Dictyostelium myosin and actin, the cells were disrupted in a Potter homogenizer, in a buffer containing 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1 mM DTT (Buffer 1), and 30% (w/v) sucrose. KCl was then added to a final concentration of 0.1 M. The homogenate was centrifuged for 1 hour at 23,000 × g. The supernatant fluid was collected and dialyzed overnight against Buffer 1 with 0.1 M KCl, and centrifugation was then repeated to collect the actomyosin pellet. This pellet was solubilized in Buffer 1 containing 0.6 M KI, 5 mM ATP, 5 mM $MgCl_2$, and 2 mM $CaCl_2$. The suspension was clarified by high speed centrifugation, and the supernatant was applied to a gel

filtration column (Bio-Gel A-15 m, equilibrated with Buffer 1 containing 0.6 M KCl and 0.2 mM ATP). The myosin obtained from this column was highly purified and was used for the studies described in this report. The actin obtained from the Bio-Gel A-15 m column was further purified by ammonium sulfate precipitation followed by a cycle of polymerization and depolymerization. The purification of these two proteins is described in greater detail elsewhere (7, 8).

For examination of Dictyostelium membranes and membrane-associated proteins, the cells were disrupted by freezing in liquid nitrogen. The membranes were collected by centrifugation and layered on a discontinuous sucrose gradient (35%/55% sucrose, with 10 mM Tris-HCl, pH 7.5, and 0.1 M KCl throughout). Most of the membrane banded at the 35%/55% boundary, after 3 to 20 hours of centrifugation at 12,000 X g.

Muscle actin, HMM, and S1 were prepared as described elsewhere (25, 26).

Analytical Methods

Electrophoresis on SDS polyacrylamide gels was carried out as described by Davies and Stark (10); the gels were stained with Coomassie Brilliant Blue. Protein concentration was determined by the method of Lowry et al (11), as modified by Hartree (12). ATPase activity was measured using [γ-^{32}P] ATP, the released P_i was collected by precipitation (13) and quantitated using a Nuclear Chicago gas-flow counter.

Samples for electron microscopy were prepared according to the procedures of Huxley and Zubay (14) and Huxley (1). Decoration of actin filaments with myosin was carried out as described by Moore et al. (15). Preparations were examined in a Philips 300 electron microscope

RESULTS

Purification and Properties of Dictyostelium Actin and Myosin

We found that actomyosin can be easily purified from Dictyostelium amoebae by virtue of its solubility in sucrose. The Dictyostelium actomyosin remained soluble in an extract prepared in 0.1 M KCl and 30% sucrose, and was selectively precipitated from this extract when the sucrose was removed. Actin and myosin were then separated by depolymerizing the actin in 0.6 M KI, and subjecting the proteins to gel filtration (Fig. 3). Highly purified myosin was recovered from the gel filtration column. The actin from this column was purified to electrophoretic homogeneity by ammonium sulfate fractionation followed by a cycle of polymerization and depolymerization.

We found that Dictyostelium myosin closely resembles muscle myosin. It consists of two heavy chains of about 210,000 daltons and two classes of light chains, about 18,000 and 16,000 daltons. In low ionic strength solutions containing Mg^{2+} the myosin molecules associate to form bipolar thick filaments (Fig. 4B) which are similar in appearance to those of muscle myosin. These thick filaments are 0.6 to 0.8 μm in length; structures with similar dimensions are seen in thin sections of Dictyostelium amoebae (Fig. 4A).

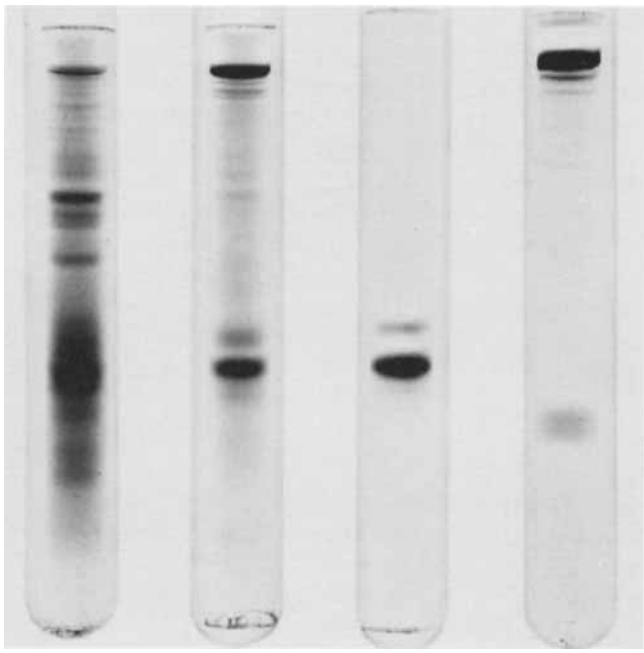

Fig. 3. Purification of Dictyostelium myosin and actin. These are SDS-acrylamide gels of Dictyostelium fractions prepared as described in Methods section. (a) Amoeba extract, 40 μg; (b) actomyosin pellet, 30 μg; (c) actin from the gel filtration column, 10 μg; (d) myosin from the gel filtration column, 10 μg.

We have also shown that Dictyostelium myosin interacts with actin, physically and enzymatically (7). It decorates filaments of Dictyostelium actin to form arrowheads. It possesses ATPase activity which is stimulated 9-fold by addition of either muscle actin or Dictyostelium actin; the specific activity of the actin-activated enzyme is 0.09 μmole P_i/min/mg myosin.

Actin purified from Dictyostelium amoebae bears a very close resemblance to muscle actin, as is also true of actin isolated from other nonmuscle cells (3). Woolley (6) showed that Dictyostelium actin forms 2-start helical filaments that are about 70 Å in

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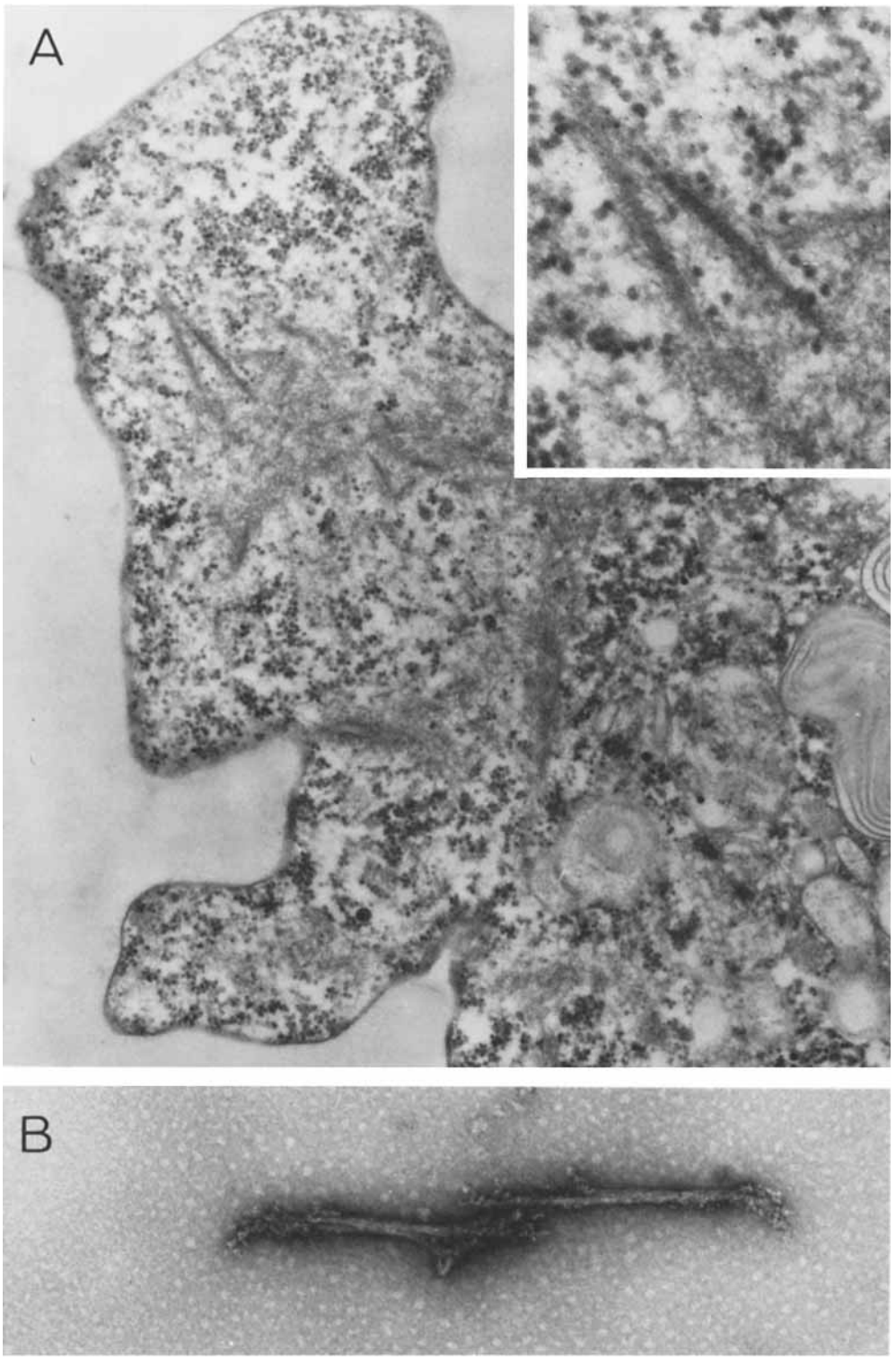
Fig. 4. (A) Thin section of a Dictyostelium amoeba and (B) negatively stained thick filaments of Dictyostelium myosin. (A) Dictyostelium amoebae were allowed to attach to a glass cover slip and were fixed for 1 hr at 25° in a phosphate buffer (0.066 M phosphate, pH 6.5, 1.5 mM $MgCl_2$ and 1.5 mM $CaCl_2$) containing 1.5% glutaraldehyde. The cells were post-fixed for 1 hr at 4° in the same phosphate buffer containing 2% osmium tetroxide, and then embedded in epoxy resin according to the method described by Luft (27). The sample was prepared and photographed by Ms. Patricia Phelps. Magnification 29,000 ×; insert is 75,000 ×. (B) Dictyostelium myosin (2 mg/ml in 0.6 M KCl) was diluted 1:20 with 10 mM $MgCl_2$, left for 2 hr at 4°, then negatively stained with 1% uranyl acetate. Magnification 75,000 ×.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diameter, and that these filaments are polar, as revealed by the arrowheads obtained by decoration with HMM. We found that Dictyostelium actin filaments form paracrystals in the presence of Mg^{2+}; similar structures are formed by muscle actin (16) and platelet actin (17). In contrast to Woolley's initial report, we found that Dictyostelium and muscle actin are identical in size, as judged by co-electrophoresis on SDS acrylamide gels (8).

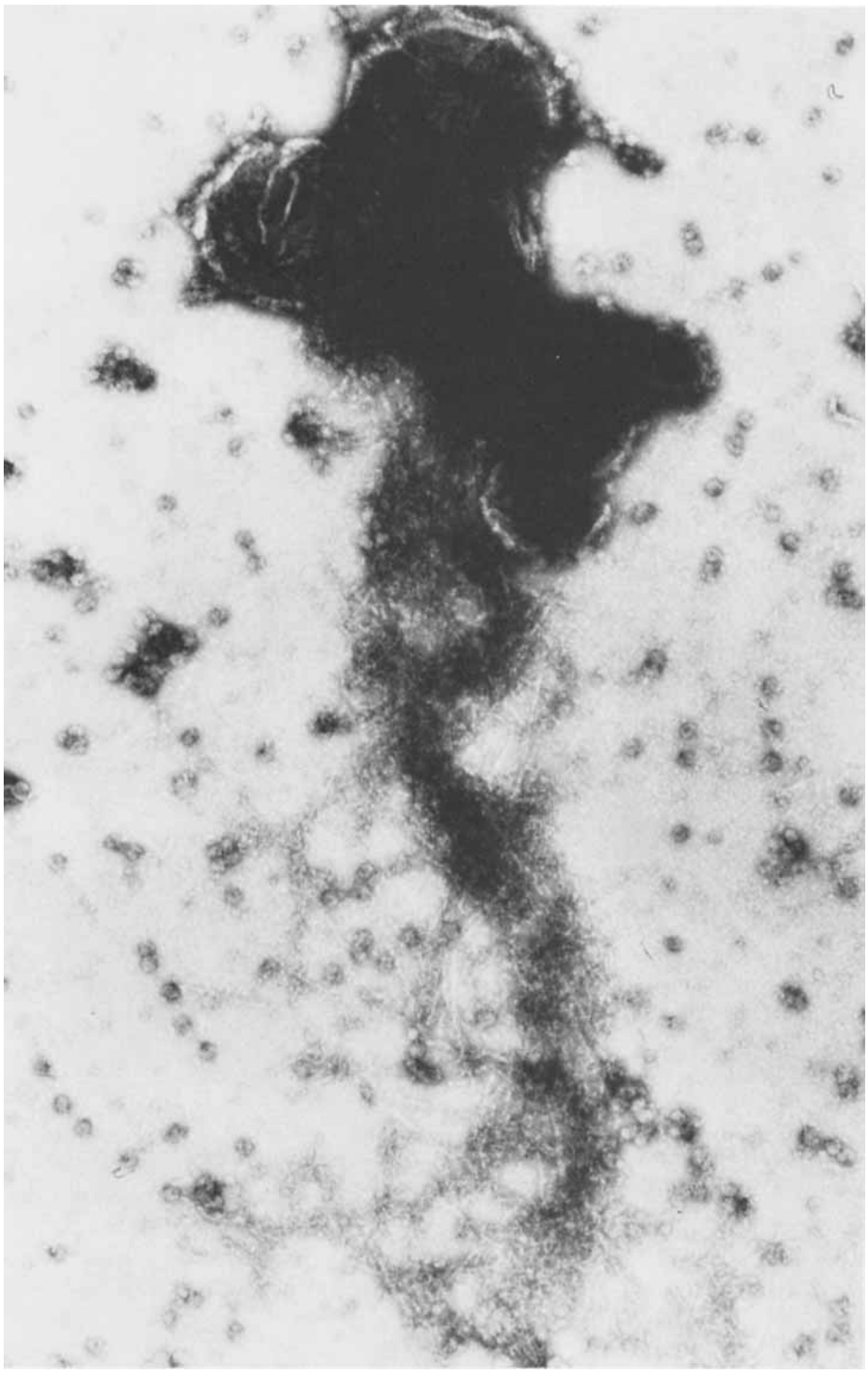
Another fundamental property of actin is its ability to activate myosin ATPase activity. Dictyostelium actin activates Dictyostelium myosin ATPase to the same extent as does muscle actin, and it is nearly as effective as muscle actin in its ability to activate muscle HMM ATPase activity (0.05 – 0.10 μmole P_i/min/mg actin, using 0.1 mg/ml HMM).

Membrane Association of Dictyostelium Actin

If actin and myosin actually are involved in motile functions of the amoeboid cell, it seems likely that some attachment must exist between these proteins and the amoeba membrane. We have observed filaments which extend from membrane fragments obtained by gentle lysis of Dictyostelium amoebae, and these filaments have dimensions similar to those of actin (Fig. 5). Upon isolation of amoeba membranes on a sucrose gradient, we found that about 15% of the Dictyostelium actin was associated with the membrane fraction. Myosin was also found in this fraction, but was not present if the membranes were isolated in the presence of MgATP, which dissociates actin and myosin. In the presence of MgATP, less actin was found with the bulk of the membrane, indicating that some actin was released when the myosin link was broken, and suggesting that the released actin either was not directly associated with membrane or was associated with small membrane fragments or vesicles which would not band on the gradient with the bulk membrane (Fig. 6). It therefore appeared that some actin is directly associated with the membrane and that in the absence of MgATP, actin-myosin interaction links myosin and more actin to the membrane (Fig. 7).

These experiments used the characteristic mobilities of actin and myosin on SDS gels to identify these components. In order to verify that the protein identified as actin by this criterion was in fact actin, we eluted this component from the membrane and characterized it. Treatment of the isolated membrane with 0.5% Triton X-100 removed all of the major polypeptides except for this component and another component of higher mobility. The putative actin was then eluted from the membrane with 0.6 M KI (Fig. 8). and was confirmed to be actin by the demonstration that it activated HMM ATPase to the extent expected for Dictyostelium actin (0.08 μmole P_i/min/mg actin, using 0.1 mg/ml HMM). In keeping with these biochemical results, examination of Triton-treated membranes by electron microscopy showed 80 Å filaments (Fig. 9); these filaments were no longer present after KI extraction.

Fig. 5. Dictyostelium membrane with associated filaments. Dictyostelium amoebae in log phase of growth were washed with 0.1 M KCl, concentrated by centrifugation, and stirred for 1 day at 4° in 10 mM potassium phosphate, pH 7, 0.1 M KCl, 10 mM $MgCl_2$ and 30% (w/v) sucrose. A drop was applied to an electron microscope grid which was then rinsed with 1% uranyl acetate; magnification 74,000 ×.



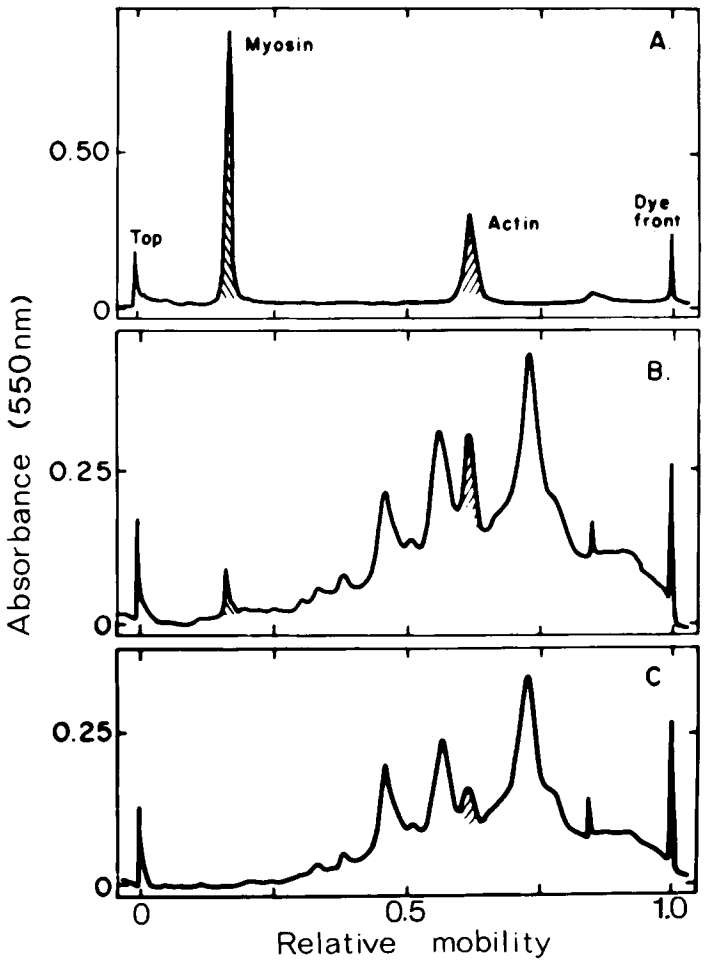


Fig. 6. Association of actin and myosin with Dictyostelium membranes. Dictyostelium membranes were prepared as described in Methods; the sucrose gradients were centrifuged at 12,000 × g for 3 hr. Membrane samples were electrophoresed on 5% SDS acrylamide gels; the gels were scanned with a Beckman Acta CIII spectrophotometer. (A) A mixture of 3 μg of Dictyostelium myosin and 2 μg of muscle actin, used as standards. (B) Dictyostelium membrane isolated in the absence of MgATP. (C) Dictyostelium membrane isolated in the presence of 5 mM $MgCl_2$ and 5 mM ATP. Gels (B) and (C) each contained 20 μg of protein.

DISCUSSION

The supramolecular structures formed by Dictyostelium actin and myosin and the interactions of these proteins with one another are very similar to their muscle counterparts. A key property of the Dictyostelium system, which may prove to be universal in nonmuscle cells, is the indication that the actin, but not the myosin, is membrane-

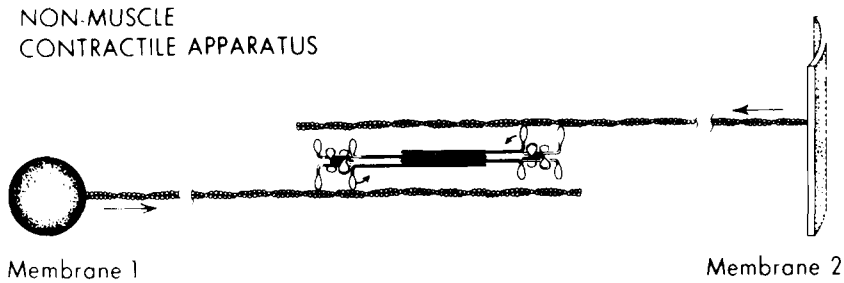


Fig. 7. A model for the interaction of membrane-associated actin with myosin to produce oriented movement in nonmuscle cells. The essential features of the model are the attachment of actin filaments to the membrane and the bipolar nature of the myosin aggregates. This model is discussed in detail elsewhere (8).

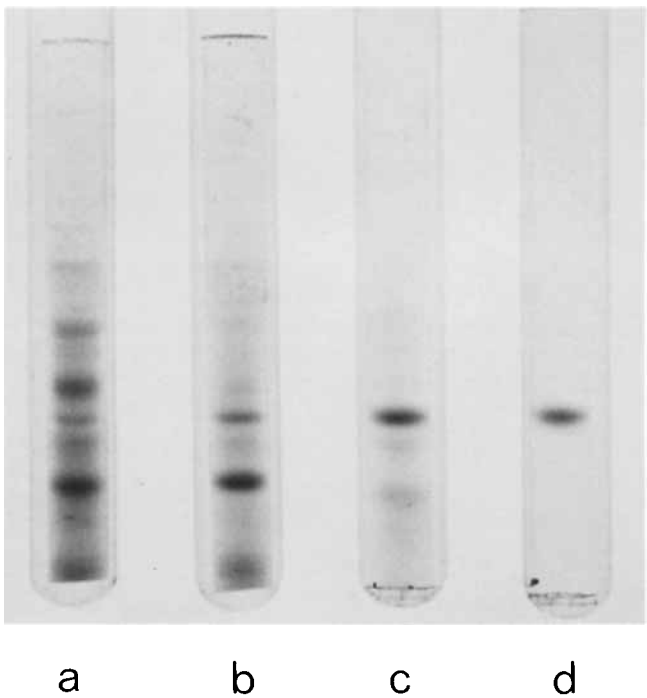
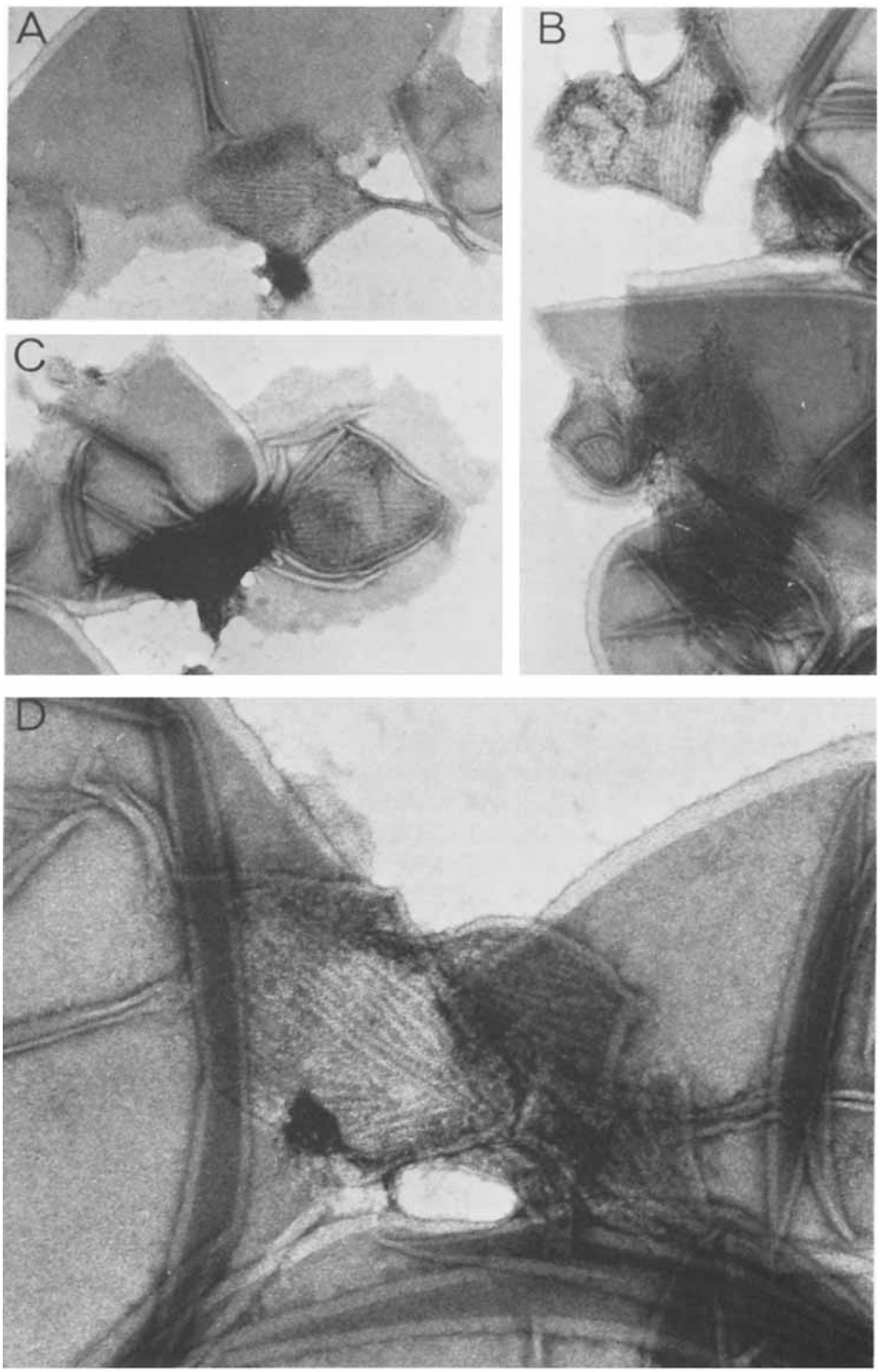


Fig. 8. Elution of actin from Dictyostelium membranes by treatment with KI. Membranes were prepared as described in Methods, with 0.2 mM MgATP present throughout the isolation procedure. The membrane pellet was resuspended in 1% Triton X-100, and after 12 hr was collected again by centrifugation. This Triton-pellet was resuspended in a buffer containing 0.6 M KI and 0.2 mM MgATP. Insoluble material was removed by centrifugation. The KI supernatant fraction contained actin which was further purified and characterized as described in the text. (a) Dictyostelium membranes, 30 μg; (b) Triton pellet, 8 μg; (c) KI solubilized protein from the Triton pellet, 5 μg; (d) muscle actin, 2 μg.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associated. Many electron microscope studies suggest that actin is localized near the plasma membrane in a variety of types of cells (e.g., 18, 19), and Pollard and Korn (4) have described evidence for association of actin filaments with the plasma membrane of Acanthamoeba castellanii.

Membrane association of filaments may serve several important biological functions. The membrane, like the Z-line in the muscle sarcomere, may act as an anchor point for actin filaments; the resulting complex could be utilized to produce contractile movement analogous to that in muscle. Assuming that the myosin heads interact only with actin filaments of appropriate polarity (a situation which is necessitated by the ordered structure of the muscle sarcomere, but which may also be generally true of these filaments; cf Figs. 1 and 7), then this arrangement would provide a mechanism for moving membrane-bounded structures toward one another. Figure 7 illustrates the unidirectional movement of membrane 1 toward membrane 2 by this mechanism. If membrane 1 were a vesicle and membrane 2 were the plasma membrane, the vesicle would be pulled to the cell surface to release its contents.

Another class of possible functions for membrane-associated actomyosin are those resulting from the linkage of actin receptor molecules in the membrane to one another. An actomyosin network on the inner surface of the membrane might provide a means of "communication" between those molecules to which it was attached, as well as restrict their mobility. This structural arrangement could also determine the shape of the cell. It has been postulated that a fibrillar network serves these functions in the erythrocyte (20, 21).

The properties of muscle actomyosin are compatible with this type of structural role. It is known that muscle actomyosin can exist in several states (relaxation, contraction, and rigor), depending on the local concentrations of Ca^{2+}, Mg^{2+}, and ATP (for review, see 22). MgATP complex is required for relaxation; Ca^{2+} can affect the level of this complex by competing with Mg^{2+} for binding to ATP. At low MgATP concentration, the actin and myosin form a rigid rigor complex. Such a complex could determine cell shape, with local relaxation being required for pseudopod formation.

It is also possible that a noncontractile structural framework is formed by actin filaments alone, linking molecules embedded in the lipid bilayer. This type of arrangement is suggested by the electron micrographs shown in Fig. 9. Negatively stained Dictyostelium membrane exhibits areas 0.1 to 1 μm wide which contain parallel filaments about 80 Å in diameter, spaced at 160 Å intervals. Further studies, including examination of thin sections and freeze-etch preparations of these membrane fractions, are required to ascertain the true significance of this filament pattern. However, these filaments are of the proper dimensions to be actin, they are not seen in membranes from which actin has been removed by KI extraction, and they are spaced at intervals which suggest that they are associated not with one another but with components of the membrane surface. The most striking aspect of these filamentous arrays was that they always appeared to lie in

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Fig. 9. Triton-treated Dictyostelium membranes. A Triton-pellet, prepared as described in Fig. 8, was negatively stained with 1% uranyl acetate; magnification 58,000 × for (A) through (C), and 110,000 × for (D).

the plane of the membrane and be bounded by it; no filaments extending free of membranes were ever seen.

Actin filaments associated with the membrane in this manner would have the potential for linking via myosin to other actin filaments attached to cytoplasmic structures. Thus a mechanism for movement similar to that presented in Fig. 7 could still apply.

Related concepts of nonmuscle movement have been discussed by various investigators who are working with other motile systems (4, 23, 24).

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