Myosin in Polymorphonuclear Leukocytes*

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SUMMARY

Myosin was extracted from guinea pig polymorphonuclear leukocytes (granulocytes) homogenized in either 0.6 M KCl or 0.34 M sucrose solutions. It was purified by precipitation at low ionic strength, ammonium sulfate fractionation, and gel filtration with buffers containing KI and ATP. Myosin isolated from sucrose extracts of granulocytes consisted of essentially one polypeptide that co-migrated with the heavy chain of skeletal muscle myosin on sodium dodecyl sulfate acrylamide gel electrophoresis. Antibody against this protein was prepared in rabbits and reacted with it to yield one precipitin line by immunodiffusion. Myosin isolated from extracts of cells prepared with 0.6 M KCl was heterogeneous by electrophoresis and immunodiffusion probably because of partial degradation by lysosomal enzymes solubilized by KCl but not by sucrose. All myosin preparations reacted with antisera to yield at least two arcs on immuno-electrophoresis. Granulocyte myosin, like other myosins, had EDTA- and calcium- but not magnesium-activated ATPase activity in 0.6 M KCl, but not by sucrose. All myosin preparations were killed, and the exudate cells were washed out of the peritoneal cavities with 0.15 M NaCl (5). Sixteen to twenty hours later the animals were killed, and the exudate cell pellets were washed twice with 10 to 15 volumes of distilled water, mixed by inversion for 30 s, and immediately centrifuged at 100,000 x gmax for 1 hour in a Beckman model L-2 ultracentrifuge. The supernatant fluids were collected for purification of myosin. In initial experiments 20 to 30 ml of packed granulocytes were suspended in 2 volumes of a solution containing 0.6 M KCl, 10 mM dithiothreitol, 2.5 mM ATP, 20 mM Tris-maleate buffer, pH 7.0, and broken in the cold room (4°C) in a Potter-Elvehjem homogenizer with a motor-driven Teflon pestle. Cell rupture was monitored by phase-contrast microscopy and was complete after 60 to 90 s. In later experiments, packed granulocytes were suspended in 2 volumes of ice-cold 0.34 M sucrose, 10 mM dithiothreitol, 2 mM ATP, 10 mM Tris-maleate, pH 7.0. The cells were disrupted in a chilled Dounce homogenizer with a tight fitting pestle. The homogenates prepared by either technique were clarified by centrifugation at 100,000 x gmax for 1 hour in a Beckman model L-2 ultracentrifuge. The supernatant fluids were collected for purification of myosin.

Preparation of Granulocyte Myosin The extract solutions

divalent cations are intimately involved with the mechanism of phagocytosis, as they are with the activation of muscle contraction, and are consistent with the hypothesis that these ions activate a contractile mechanism (1). The discovery of actin and myosin in non-muscle cells suggests that many forms of cellular and subcellular movement may be similar at the molecular level (2). Extracts of horse peripheral blood mixed leukocytes which include granulocytes have properties consistent with the presence of these contractile proteins (3, 4). To explore the basis of cellular motion involved in phagocytosis, we have purified myosin from polymorphonuclear leukocytes. This report describes the procedure for the isolation of granulocyte myosin, some electrophoretic, immunochemical, and ultrastructural properties of this protein, and aspects of its interaction with divalent cations and with skeletal muscle actin and regulatory proteins.

METHODS

Granulocytes—Acute peritoneal exudates were induced in guinea pigs. Twenty to thirty female Hartley strain guinea pigs weighing 400 to 500 g each received intraperitoneal inoculations of 15 ml of autoclaved sodium caseinate, 120 mg per ml in 0.15 M NaCl (5). Sixteen to twenty hours later the animals were killed, and the exudate cells were washed out of the peritoneal cavities with 0.15 M NaCl (25°C). The cell suspensions were thereafter kept at ice bath temperature and were washed thrice with 10 to 21 volumes of 0.15 M NaCl by centrifugation at less than 100 x g. After the last wash the cell pellets were suspended in 10 to 15 volumes of distilled water, mixed by inversion for 30 s, and immediately centrifuged at 250 X g. Preparation of Granulocyte Myosin The extract solutions

Phagocytosis, the major function of polymorphonuclear leukocytes, is characterized by active ameboid motion. Kinetic studies of the ingestion of particles by granulocytes reveal that

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† Established Investigator of the American Heart Association.
‡ The abbreviations used are: granulocyte, polymorphonuclear leukocyte; SDS, sodium dodecyl sulfate; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N′-tetraacetic acid.
were dialyzed overnight at 4° against three changes of 15 volumes of 0.05 M KCl, 5 mM dithiothreitol, 20 mM Tris-maleate buffer, pH 7.0, which resulted in the formation of a precipitate. The precipitate was collected by centrifugation at 20,000 × g and dissolved in 3 to 4 volumes of 0.6 M KCl, 10 mM dithiothreitol, 20 mM Tris-maleate buffer, pH 7.0 (Buffer A). This solution was fractionated with saturated ammonium sulfate containing 0.01 M EDTA, precipitates being collected at saturations of 0 to 20, 20 to 50, and 50 to 70%. K⁺- and EDTA-activated ATPase activity was invariably found in the 20 to 50% fraction. The precipitates of this fraction were dissolved in 0.6 M KI, 6 mM sodium thiosulfate, 10 mM dithiothreitol, 0.5 mM MgCl₂, 0.5 mM ATP, 20 mM Tris-maleate buffer, pH 7.0, and dialyzed for 2 hours against the same solution. The dialyzed solution was applied to a column, 1.5 × 72 cm, of Bio-Gel A-15M (4% agarose) in Buffer A, and concentrated in a collodion bag under vacuum. This material was designated granulocyte myosin. It was stored in Buffer A at 4° or at −20° after purification with 50% saturated ammonium sulfate.

Preparation of Proteins from Muscle—Back and leg muscles were removed from New Zealand albino rabbits. F-actin and a troponin tropomyosin complex were prepared as described by Spudich and Watt (8). Myosin A was obtained by the technique of Kielley and Bradley (7).

Assay of ATPase Activity—Routine assays were made in 1 ml containing 0.6 M KCl, 1.1 mM ATP, 15 mM Tris-maleate buffer, pH 7.0, with either 2 mM EDTA, 5 mM CaCl₂, or 5 mM MgCl₂, and are referred to as K⁺- and EDTA-activated, Ca²⁺-, and Mg²⁺-ATPase activity, respectively. The conditions for measurement of actin activation of granulocyte myosin ATPase were 0.06 M KCl, 1.1 mM ATP, 5 mM MgCl₂, 20 mM Tris-maleate, pH 7.0. Either 0.2 mM CaCl₂ or 0.5 mM EGTA were added as indicated. After incubation at 25° for 20 to 60 min, the ATPase reaction was stopped with 0.5 ml of ice-cold trichloroacetic acid (15 mg per ml), the tubes chilled, and the precipitated protein removed by centrifugation. Orthophosphate was measured in 1 ml of the supernatant fluid by the technique of Fiske and SubbaRow (8). Under the assay conditions generation of orthophosphate was constant with time and proportional to myosin concentrations. Protein was assayed by the Folin method (9) with bovine albumin as the standard.

SDS Acrylamide Gel Electrophoresis—Protein samples were prepared by dialysis against distilled water, and lyophilized. The dried proteins were dissolved in a solution containing 8 M urea, 50 mM dithiothreitol, 10 mg per ml of SDS, 0.1 M sodium phosphate buffer, pH 7.0, sucrose, 100 mg per ml, and bromphenol blue, 5 mg per ml, incubated in that solution at 37° for 2 hours and briefly heated at 100°. The denatured proteins were subjected to electrophoresis for 6 to 8 hours at 6 to 8 ma per gel on SDS gels (Fig. I). Definite but variable purification of peptides which co-migrated with skeletal muscle myosin and actin on SDS gels (Fig. I). Definite but variable purification of actin and a troponin tropomyosin complex were prepared as described by Spudich and Watt (8). Myosin A was obtained by the technique of Kielley and Bradley (7).

RESULTS

Granulocyte Preparations—Wright's stained smears of the cells showed 90 to 95% mature polymorphonuclear leukocytes. The remainder of the cells were band forms, lymphocytes, monocytes, and rarely eosinophils. Platelets or erythrocytes were not seen.

Isolation of Granulocyte Myosin—We used two methods to detect myosin during its purification: (a) ATPase assays in concentrated KCl, in which myosin ATPase activity has the unique property of being inhibited by Mg²⁺ and activated by both Ca²⁺ and EDTA (14); and (b) polyacrylamide gel electrophoresis in SDS, which reveals polypeptides with high molecular weight (200,000) typical of myosins from muscle and non-muscle cells of higher organisms.

Extracts of granulocytes prepared with either 0.6 M KCl or 0.34 M sucrose containing ATP and dithiothreitol had K⁺- and EDTA-activated ATPase activity (Table I) and contained poly-peptides which co-migrated with skeletal muscle myosin and actin on SDS gels (Fig. I). Definite but variable purification of the enzyme activity and the poly-peptides was achieved by low ionic strength precipitation. The recovery of ATPase and protein was greater if the ionic strength was lowered by dialysis rather than dilution. Further purification was obtained by ammonium sulfate fractionation (Fig. I), with the majority of the enzyme activity invariably being found in the fraction precipitated between 20 and 50% saturation.

The most important step in the purification was chromatography of the 20 to 50% ammonium sulfate fraction on Bio-Gel A-15M (4% agarose) in a buffer containing 0.6 M KI with ATP.

Light Scattering—Solutions of myosin (0.1 ml) were mixed with various buffers (0.9 ml) in quartz cuvettes maintained at 28° in a water-jacketed carrier. Optical density changes with time at 350 nm were measured with a Gilford spectrophotometer and recorder.

Electron Microscopy—Myosin samples were applied to carbon- and Formvar-coated grids made hydrophilic by glow discharge and negatively stained with 1% uranyl acetate as described previously (11). The grids were examined in a Siemens Elmiskop 101 with an accelerating voltage of 100 kv. Magnifications were calibrated using negatively staining paracrystals of muscle tropomyosin with a periodicity of 395 Å.

Preparation of Antisera and Immunochimical Analysis—Purified granulocyte myosin was emulsified with complete Freund's adjuvant, and 20 to 40 μg of the protein were injected beneath the dorsal skin of rabbits. Booster injections of 20 to 40 μg were given every 2 weeks until precipitating antibodies were detected, usually after 6 to 8 weeks. Double diffusion was performed on microscope slides containing 1% agar gel prepared with 0.3 M KCl solution containing 15 mM sodium phosphate buffer, pH 7.0, and 0.5 mM EDTA by the Ouchterlony procedure (12). Immunelectrophoresis was performed with the method of Scheidegger (13) with the same agar gel preparations used for immunodiffusion. Myosin was subjected to electrophoresis at 6 ma for 6 hours and then reacted against antiserum in a humidified atmosphere at 37° overnight. All slides were then washed in 0.15 M NaCl, 15 mM phosphate buffer, pH 7.0, stained with 1% Amido black in 5% acetic acid and 50% methanol, and destained with 5% glycerol, 2% acetic acid in 50% methanol.

Materials—Crystalline bovine serum albumin was purchased from Armour, catalase, phosphorylase a, trypsin, bovine albumin, and ovalbumin from Worthington; sodium caseinate from Difco; dithiothreitol, disodium ATP, and β-galactosidase from Sigma. All solutions were prepared with deionized water.
TABLE I

Purification of granulocyte myosin

Purification of myosin extracted by the 0.6 M KCl method from granulocytes. Details of the procedure are found in the text and Fig. 2. ATPase activity was measured in 0.6 M KCl at 25°C under standard conditions.

| Total protein | Total ATPase activity | Specific ATPase activity |
|---------------|-----------------------|--------------------------|
|               | mg                    | K⁺-EDTA | Ca²⁺ | Mg²⁺   | K⁺-EDTA | Ca²⁺ | Mg²⁺   |
| Whole homogenate | 1,920                | 2,862   | 8,049 | 11,145 | 2.6     | 7.8  | 10.8   |
| Extract supernatant | 1,032                | 1,062   | 1,809 | 912    | 6.4     | 10.9 | 5.5    |
| Low ionic strength precipitate | 165            | 630     | 660   | 159    | 17.0    | 17.9 | 4.3    |
| 20 to 50% ammonium sulfate fraction | 36            | 2,4     | 550   | 24     | 186     | 104  | 10     |
| Purified myosin from Bio-Gel A-15M column | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 |

As shown in Fig. 2, the K⁺- and EDTA-activated ATPase activity was recovered in the first protein peak which eluted from the column with approximately the same partition coefficient as muscle myosin. This fraction was termed purified granulocyte myosin. If the ammonium sulfate fraction was dissolved in 0.6 M KCl with ATP and chromatographed in the same buffer, the granulocyte myosin fractions were always contaminated with varying amounts of actin as revealed by electrophoresis and by electron microscopy. The 0.6 M KCl-ATP buffer depolymerized the granulocyte actin so that it could be separated from the myosin on the gel filtration column.

The yield of myosin ranged from 1.2 to 7.8 mg per g of homogenate protein, with a mean of 3.2 mg per g for nine isolations. Therefore the myosin comprised at least 0.89% of the cells total protein. Scans of SDS acrylamide gels of whole granulocyte homogenates revealed that myosin comprised about 1.5% of the stainable protein. Since these extracts contained approximately 50% of the total cellular protein, the estimates of the myosin composition of the granulocytes obtained from yields during purification and from the gels were comparable. Although actin was not purified from the granulocyte extracts, scans of SDS acrylamide gels of whole granulocyte homogenates revealed that a protein with the same mobility as muscle actin comprised about 10% of the total homogenate protein.

**Purity and Subunit Composition**—Purity and subunit composition of purified granulocyte myosin were assessed by electrophoresis on polyacrylamide gels in the presence of SDS. Most preparations made by gel filtration in KI and ATP consisted of one major band which co-migrated with the heavy chain of muscle myosin (mol wt 200,000) and which accounted for 93% of the stained protein on the gel (Fig. 3). The only other bands were in the 20,000 molecular weight region and may be low molecular weight components of the granulocyte myosin. Some preparations of myosin purified from KCl extracts (but not from sucrose extracts) consisted of two or more protein bands in the 180,000 to 200,000 molecular weight range. As shown in Fig. 4, the protein in these bands would bind to actin filaments like the 200,000-dalton component (see below) suggesting that they were partially degraded myosin heavy chains.

**ATPase Activity**—Like other myosins, granulocyte myosin had ATPase activity which was inhibited by Mg²⁺ and activated by Ca²⁺. 

![Fig. 2. Chromatography of the 20 to 50% saturated ammonium sulfate fraction on a column (1.5 X 75 cm) of Bio-Gel A-15M, 100 to 200 mesh, in 0.6 M KI, 10 mM sodium thiosulfate, 10 mM dithiothreitol, 0.5 mM MgCl₂, 0.5 mM ATP, and 20 mM Tris-maleate, pH 7.0. Fractions of 1.6 ml were collected. The ordinates indicate absorbance of fractions of 290 nm (●) and K⁺- and EDTA-activated ATPase (○). The void volume (V₀) and total volume (V₀ + Vₐ) are indicated with arrows.](http://www.jbc.org/)

![Fig. 1. SDS polyacrylamide gel electrophoreograms of: left, granulocyte extract prepared with 0.34 M sucrose, 10 mM dithiothreitol, 2 mM ATP, and 10 mM Tris-maleate, pH 7.0; middle, granulocyte extract prepared with 0.6 M KCl, 10 mM dithiothreitol, 2.5 mM ATP, and 20 mM Tris-maleate, pH 7.0; right, 20 to 50% saturated ammonium sulfate fraction. The two gels on the left were prepared with 5% acrylamide; the gel on the right with 10% acrylamide. The numbers indicate the molecular weights of proteins comigrating with skeletal muscle myosin and actin.](http://www.jbc.org/)

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**ATPase Activity**—Like other myosins, granulocyte myosin had ATPase activity which was inhibited by Mg²⁺ and activated by Ca²⁺ or by EDTA with high concentrations of K⁺, but relative to other myosins, the specific activity of the granulocyte myosin ATPase was low. The K⁺- and EDTA-activated
Interaction with Muscle Regulatory Proteins—The actin-activated granulocyte myosin ATPase activity was not significantly influenced by the presence of EGTA or calcium ions (Table III). However, addition of skeletal muscle troponin-tropomyosin to the assay made the actin-activation dependent upon the presence of low concentrations of Ca$^{2+}$. In the absence of Ca$^{2+}$ actin activation was completely inhibited.

Antibodies to Granulocyte Myosin—As shown in Fig. 7, antiserum to granulocyte myosin purified from sucrose extracts yielded a single major precipitin line by double diffusion in agar gel, although occasionally with preparations purified from KCl extracts of the cells, a second minor band was seen. The antiserum reacted weakly with rabbit skeletal muscle myosin (Fig. 7). At least two arcs were detected in all preparations by immunoelectrophoresis (Fig. 8).

Filamentous Aggregates of Granulocyte Myosin—In low ionic strength buffers in the presence of divalent cations, granulocyte myosin spontaneously self-assembled into bipolar filamentous aggregates (Fig. 5B). The granulocyte myosin filaments were very small being about 250 nm long and 6 to 12 nm wide. These myosin aggregates formed over a period of several minutes when myosin dissolved in 0.6 M KCl was diluted to 0.1 M KCl. Dissipation of monomeric myosin against 0.1 M KCl with divalent cations also resulted in filamentous aggregates. Like other myosins, these assemblies of granulocyte myosin were rapidly broken down when the ionic strength was increased to greater than 0.4 M with KCl.

We have not made an extensive study of the divalent cation requirement for filament formation. However, it was clear that EDTA inhibited aggregation and low concentrations of either Ca$^{2+}$ or Mg$^{2+}$ promoted aggregation. The EDTA did not simply denature the myosin, because solutions of myosin in 0.1 M KCl contained no filaments, but contained normal appearing bipolar aggregates if divalent cations, in excess of the EDTA, were added.

Like other myosin filaments, granulocyte myosin filaments had a bare fibrous shaft in the center and globular projections at both ends. These projections contain the actin binding sites of the myosin as shown in Fig. 5C, where several myosin aggregates have bound to actin filaments by the globular projections. The bare central shaft contained no actin binding sites, but formed an important part of the cross-links made between actin filaments by the bipolar myosin aggregates. Even in the presence of EDTA there was some tendency for the granulocyte myosin to form filaments when associated with actin filaments.

Changes in the turbidity of myosin solutions correlated with the morphological observations (Fig. 9). Granulocyte myosin had a higher absorbance in 0.1 M KCl than in 0.6 M KCl, and there was a time-dependent further increase in absorbance when divalent cations were added to the myosin in 0.1 M KCl. A constant and puzzling finding was a marked increment in absorbance with visible turbidity following the addition of EGTA to myosin solutions containing CaCl$_2$. EGTA did not have this effect in the absence of calcium, even with Mg$^{2+}$ present. Electron microscopy of the precipitate revealed some myosin filaments tangled in amorphous aggregates. Similar precipitates were observed in the 20 to 50% ammonium sulfate fraction after treatment with 0.1 mM CaCl$_2$-EGTA.

**DISCUSSION**

The isolation of actin and myosin from diverse cells has made it imperative to deal with uniform cell populations when particular contractile proteins are to be described. The guinea pig...
Fig. 5. Electron micrographs of negatively stained granulocyte contractile proteins. A and B, bipolar filaments formed from purified granulocyte myosin in 0.12 M KCl, 1 mM CaCl₂, and 10 mM imidazole, pH 7.0. C, granulocyte myosin bound to granulocyte actin filament in 0.12 M KCl, 0.5 mM EDTA, and 10 mM imidazole, pH 7.0. There is some tendency for the myosins to aggregate along the sides of the decorated filament. D and E, same preparation as C. In some cases, such as these, the myosin can cross-link actin filaments, although there is no tendency for the myosin to form bipolar filaments by itself. Magnification × 100,000.

Peritoneal exudates induced acutely with sodium caseinate yield large quantities of mature polymorphonuclear leukocytes (5). Less than 5% of the cells are lymphocytes, monocytes, and macrophages. We employed low speed (less than 100 × g) centrifugations during the washing of the cells to ensure removal of platelets should they be present in the exudates. Erythrocytes were quantitatively eliminated by means of vigorous hypotonic lysis. The granulocytes did not rupture under such conditions.
FIG. 6. Effect of skeletal muscle F-actin on the Mg\textsuperscript{2+}-ATPase activity of guinea pig granulocyte myosin. The concentration of myosin in the assays was 0.24 mg per ml.

Table III

| Proteins\textsuperscript{a} | Mg\textsuperscript{2+}-ATPase activity\textsuperscript{b} |
|-----------------------------|---------------------------------|
|                             | + EGTA (0.5 mM) | + CaCl\textsubscript{2} (0.2 mM) |
| Myosin                     | 2.5              | 3.5                        |
| Myosin + F-actin           | 7.6              | 7.4                        |
| Myosin + F-actin + troponin-tropomyosin | 2.8              | 8.8                        |

\textsuperscript{a} The proteins were present in the incubation solutions in the following concentrations: granulocyte myosin, 0.24 mg per ml; muscle F-actin, 0.68 mg per ml; muscle troponin-tropomyosin, 0.62 mg per ml.

\textsuperscript{b} Mg\textsuperscript{2+}-ATPase activity was measured at a low ionic strength as described in the text.

and remained intact prior to exposure to hypertonic KCl or sucrose.

Myosin was purified from granulocyte extracts by precipitation at low ionic strength, ammonium sulfate fractionation, and gel filtration in buffers containing ATP. This general approach has been effective for purifying myosin from platelets (11) and from fibroblasts (15). As observed in the case of platelets, but not fibroblasts, granulocyte myosin obtained by chromatography in 0.6 M KCl solutions was heavily contaminated with actin. Evidence that the contaminating protein was actin included its co-migration with skeletal muscle actin on SDS polyacrylamide gel electrophoresis, the appearance of "decorated" filaments in electron micrographs of the protein solutions and the lack of activation of Mg\textsuperscript{2+}-ATPase activity of contaminated preparations by added skeletal muscle F-actin. Unlike similar preparations from platelets, however, proteins corresponding to myosin head and myosin rod were not isolated with the granulocyte myosin. When 0.6 M KI was substituted for KCl during chromatography (a procedure originally described for the purification of muscle actin (16)), actin was eliminated from the myosin preparations.

Unexpectedly, myosin was efficiently extracted from granulocyte extracts with ATP as well as by the 0.6 M KCl solutions which have been employed to extract contractile proteins from platelets and fibroblasts (11, 15). When granulocytes are homogenized in hypertonc sucrose solutions by means of the technique described, their granules remain intact (17). By this procedure, the granules which contain numerous hydrolytic enzymes (18) can be removed by centrifugation. Therefore, the myosin, when extracted by this procedure, is exposed to lower concentrations of potentially lytic enzymatic activity. In fact, myosin purified from sucrose extracts of granulocytes appeared homogenous by SDS acrylamide gel electrophoresis and immunodiffusion in contrast to some myosin preparations purified from KCl extracts. These findings suggested that partial degradation of the myosin heavy chain indeed occurred in the KCl preparations. This degradation, in addition to causing electrophoretic heterogeneity, may have revealed more antigenic sites than the sucrose preparations. However, all granulocyte myosin preparations were resolved into two arcs by immunoelectrophoresis. Immunochemical heterogeneity has been observed in some purified muscle myosins by immunodiffusion (19, 20), and its basis is not clear.

The granulocyte myosin resembled skeletal muscle myosin in many respects including the size of its heavy chains, its morphology, its unique pattern of ATPase activation, its reversible binding to actin filaments and the activation of its Mg\textsuperscript{2+}-ATPase activity by actin. Compared with myosin filaments from skeletal muscle, the granulocyte myosin filaments were very small,
but in other respects the structures were similar. Short myosin filaments are typical of some other cytoplasmic myosins, including myosin from platelets (11), fibroblasts (15), and Physarum (21).

Divalent cations had two effects of potential physiological importance: assembly of myosin filaments and regulation of myosin ATPase activity.

Calcium or magnesium stimulated the formation of bipolar myosin filaments. Calcium had similar effects on the aggregation of Physarum myosin (22) and the appearance of myosin-like filaments in tissue sections of some cells (23, 24). Since these filaments are necessary for the formation of mechanical connections between actin filaments, their assembly may be one of the steps in the activation of the granulocyte contractile machinery for movement, including phagocytosis. The small size of the granulocyte myosin filaments and the small amount of myosin in granulocytes relative to the amount of actin probably account for the apparent absence of myosin filaments in electron micrographs of these cells.

Calcium also influences the Mg$^{2+}$ATPase activity of the myosin in the presence of regulatory proteins. Because the cell has an excess of Mg$^{2+}$ over Ca$^{2+}$, and because Mg$^{2+}$ strongly inhibits myosin ATPase activity in 0.6 M KCl, the Ca$^{2+}$ activation in 0.6 M KCl solutions shown in Table I is probably not relevant physiologically. Instead the actin-activated Mg$^{2+}$ATPase is probably operational in the cell and involved in the transduction of chemical energy stored in ATP into force for movement, as it is in muscle contraction. The actin-activated Mg$^{2+}$ATPase activity of granulocyte myosin was not influenced by the presence or absence of small amounts of calcium ions (indicating that calcium regulation is an intrinsic feature of the myosin as it is for certain molluscan myosins (25)). However, the presence of the muscle regulatory proteins, troponin-tropomyosin, made the actin-activated ATPase of granulocyte myosin dependent upon the presence of calcium. Troponin-tropomyosin has not yet been isolated from any non-muscle cell, although Shibata et al. reported the isolation of proteins from horse peripheral blood leukocytes which had Ca$^{2+}$ dependent ATPase activity and superprecipitation (4). This may indicate the presence of tropolin-tropomyosin, but the meaning of their observations is obscure by the fact that actomyosin prepared by their technique is composed of numerous proteins (cf. Fig. 1), and contains considerable Ca$^{2+}$ATPase activity that does not co-purify with granulocyte myosin. In addition, our observation of precipitation of granulocyte myosin in the presence of EGTA-calcium buffers makes interpretation of their superprecipitation data (obtained by light scattering) difficult.

Assuming that the striking similarity of muscle and granulocyte myosins reflects a common mechanism of force generation, it is clear from our knowledge of muscle contraction, that the effects of divalent cations on the assembly granulocyte myosin filaments and on the enzyme activity of granulocyte myosin could regulate the molecular events leading to cell movement and perhaps explain the prominent influence that these ions have on phagocytosis.

\[ \text{Note Added in Proof—Recently Tatsumi et al. have extended their work on contractile proteins from mixed equine peripheral blood leukocytes (26) by isolating and partially characterizing actin and myosin from these cells.} \]

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