PROPERTIES OF PHYSARUM MYOSIN PURIFIED BY A POTASSIUM IODIDE PROCEDURE

V. T. NACHMIAS

From the Department of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19174

ABSTRACT

Myosin has been purified free of actin from Physarum actomyosin by a two step adaptation of the classical potassium iodide method for depolymerizing actin. On 12% sodium dodecyl sulfate (SDS) gels, the single major slowly moving protein band present in the calcium activated adenosine triphosphatase peak (90% pure) is associated with two fast moving bands of molecular weights of approximately 17,000 and 21,000 daltons, respectively. Densitometry shows the molar ratio of heavy chains to the 21,000 and 17,000 dalton chains on the gels to be 1:2:1.

The highly purified myosin forms filaments up to 2.5 μm long in the presence of 5 mM magnesium and 0.05 M KCl. Calcium ions were not required for the formation of long filaments from this highly purified myosin.

At low ionic strength (0.05 M KCl) the magnesium ATPase of the highly purified myosin is activated four- to tenfold by muscle actin. The extent of activation is a function of the actin concentration and levels off at high levels of actin. In 0.1 mM calcium salts the ATPase activity is approximately 60% of that in 1 mM EGTA.

In summary, Physarum myosin is similar to a number of muscle myosins as well as to platelet and fibroblast myosin, which all possess light chains of two different molecular weights associated with the heavy chains. Under ionic conditions close to those in vivo, highly purified Physarum myosin aggregates into long filaments.

INTRODUCTION

Actomyosins sharing some of the major properties of muscle actomyosin are now readily prepared from several nonmuscle sources including Physarum (1, 2) platelets (3, 4), Naegleria (5), leukocytes (6), Dictyostelium (7), and brain (8).

Highly purified myosin free of actin has been difficult to obtain from actomyosin largely because the actin component cannot be easily separated from the myosin.

In general, the procedures used have involved the partial separation of myosin from actin by ultracentrifugation under dissociating conditions (Mg-ATP¹ or Mg-pyrophosphate) as first demonstrated by A. Weber in 1956 (9). This procedure, coupled with gel filtration, has been used for the purification of myosin from Physarum, platelets and fibroblasts (10, 11, 12). However, actin is not completely removed. With Physarum, considerable actin remains in the supernate after ultracentrifugation. It has been removed by allowing it to

¹Abbreviations used in this paper: EGTA, ethyleneglycol bis (β-aminethyl ether) N,N-tetraaetetic acid; ATP, adenosine triphosphate; MEAM, myosin-enriched actomyosin.
recomplex with the supernatant myosin followed by precipitation of this 'myosin-enriched fraction'; free myosin remains in the supernate (13, 14). However, after gel filtration (10) the yield is only of the order of 0-0.5 mg purified myosin per 100 g myxomycete. On the other hand, the low salt method of Adelman and Taylor (15) results in very dilute protein samples and requires three column procedures.

These difficulties in preparing Physarum myosin have also been associated with very low or no actin activation (13, 15), a crucial criterion for evaluating actin-myosin interaction. Similar problems exist for other systems including vertebrate smooth muscle myosin (16). Recently, Puszkin and Berl (17) revived the use of potassium iodide (KI) (18, 19) to depolymerize and separate brain actin from a myosin component on sucrose density gradients containing ATP and KI. We have now found that a brief treatment of Physarum myosin-enriched actomyosin with 0.6 M KI in the presence of magnesium pyrophosphate permits essentially complete subsequent separation—by gel chromatography—of enzymatically active myosin from other proteins, except for a small amount of high molecular weight protein. Several of the properties of the myosin have been examined and compared with those of the supernatant myosin fraction.

MATERIALS AND METHODS

Preparation of KI Myosin

(Table 1 a.) Actomyosin was prepared from Physarum by a modification of the method of Hatano and Tazawa (1) as shown in Table 1 a (steps 1-4). A myosin-enriched fraction (MEAM) was then obtained (Table 1 a, steps 5-6).

A 3 M KI stock solution was treated with 20 mg Norit per ml to remove I₂ directly before use. MEAM precipitates (Table 1 a, step 6) were dissolved in 10 ml of 0.6 M KI, 0.05 M imidazole, 0.1 mM dithiothreitol (DTT), 2.5 mM Mg-pyrophosphate pH 7 and stirred at 5°C for 15 min (Table 1 a, step 7).

Samples were applied to a 90-cm column of Bio-Rad A-15 m (Bio-Rad Laboratories, Richmond, Calif.) pre-equilibrated with two column volumes (1 liter) of a high salt buffer (HS buffer) containing 0.5 M KCl, 0.05 M imidazole, 0.1 mM DTT, pH 7 (HS buffer) (Table 1 a, step 8). Fractions (5 ml) were collected at flow rates of ~10 ml/h. All procedures were carried out at 5°C. Absorptions were measured at 320, 280, and 260 nm in a Zeiss PMQ spectrophotometer (Carl Zeiss, Inc., New York), and absorptivity values corrected for light scattering.

Enzyme Assays

The Ca⁺⁺ K⁺-ATPase activity of fractions was estimated by a semimicro version using the exact assay conditions of Adelman and Taylor (15). Actin activation was carried out at 22-24°C in a low salt buffer (LS buffer) containing 0.03 M KCl, 0.02 M imidazole, 0.1 mM DTT, pH 6.82-6.85. The substrate was 2.5 mM Mg-ATP and the incubation time 15 or 30 min, with shaking at 120 displacements/min. Myosin and actin samples were dialyzed against LS buffer before assay.

Actin Preparation

Rabbit muscle actin was prepared from acetone powders by a single pelleting after a treatment with 0.6 M KCl as described by Spudich and Watt (20).

ACTIN PREPARATION

Rabbit muscle actin was prepared from acetone powders by a single pelleting after a treatment with 0.6 M KCl as described by Spudich and Watt (20).

V. T. NACHMIS Physarum Myosin Purified by Potassium Iodide Procedure 55
**Gel Analysis**

SDS gel electrophoresis was carried out according to Laemmli, using 13-cm gels of 10-15% acrylamide (21). Densitometry was performed on Coomassie blue-stained gels with a Canalco model K instrument (Canalco, Inc., Rockville, Md.) using a 0.02-mm slit width.

**Electron Microscopy**

Highly purified myosin from peak fractions was dialysed against 0.05 M KC1, 5 mM imidazole, 0.1 mM DTT, pH 7 with (a) 1 mM EGTA. (b) 5-10 mM MgCl2 with 1 mM EGTA, (c) 5-10 mM Mg with 0.1 mM CaCl2. Crude myosin (Table I a, step 6) was already in 0.05 M KC1; Mg-EGTA or MgCa was added to this directly to yield the same final concentrations as for purified myosin. In other experiments, crude myosin was also dialysed against (a), (b), and (c).

Samples were negatively stained on carbon-coated grids, as previously described (2) and examined in a Hitachi HU-11A microscope equipped with 50-μm thin gold-foil aperture, or in a JEM 100 with 60-μm molybdenum aperture.

**RESULTS**

**Gel Filtration**

Fig. 1 shows a typical example of a gel filtration experiment using this procedure. After the high 260 absorbing voided peak, one major well-separated peak (Kav 0.21 on two columns of different geometries) contains all the Ca-ATPase activity. 80% of the input Ca-ATPase was recovered. Peak fractions show an OD ratio 280/260 of 1.6-1.9 and the specific activity is 1.0-1.2 μmol P1/min/mg (22-24°C). These values are significant in being generally slightly higher than those previously reported (13, 14, 15, 10) or resulting from variants of this procedure. For comparison, if KI is not used, and MEAM fractions chromatographed directly on columns eluted with high KC1 plus Mg-pyrophosphate, ATPase activity appears both in the void and in a first peak. Here, however, the specific activity of the first peak ranges from 0.7 to 0.9 (little difference from the MEAM fraction itself: Table I b) while the A 280/260 is 1.3-1.4. Actin is detectable in this first peak in four out of five columns, when examined by SDS gels or by electron microscopy. KI treatment of crude actomyosin without preparation of MEAM results in a large voided peak containing the ATPase activity, and no second peak.

We conclude that chromatography of MEAM under dissociating conditions results in some actin still associated with myosin, but that with KI treatment essentially all the actin is depolymerized to appear in a later column fraction closely followed by KI and nucleotide. Estimation of protein by the Lowry procedure showed (Fig. 1) 0.2 mg/ml protein in peak 1 and about the same concentration in the shoulder (peak 2), while less than 0.01 mg/ml protein was found in fraction 165 (peak 3).

![Figure 1](image)

**TABLE I b**

Typical Yields of Myosin from 100 Grams Myxomycete

| Stage                        | Protein | Total* | Specific activity μmol P1/min/mg |
|------------------------------|---------|--------|---------------------------------|
| Crude-homogenate             | 2970    |        | 0.04                            |
| Two times precipitated       | 60      | 24     | 0.4                             |
| Myosin-enriched fraction     | 12      | 8.4    | 0.7                             |
| Column myosin                | 4.7     | 5.6    | 1.2                             |

*Crude homogenate enzyme activity assayed under these conditions includes other enzymes than myosin alone.
FIGURE 2 (a) 12% SDS gels from the 90-cm column of Fig. 1. 1: 50 μg input protein; 2: 46 μg ATPase peak fraction (Fxн) 92; 3: 23 μg ATPase peak Fxн 94; 4: est. 20 μg Fxн 145. (b) Superimposed densitometer tracings of 12% SDS gels. Upper trace: Myosin fraction from another experiment containing approximately 10 μg actin in peak marked A. Lower trace: Fig. 2 (a), no. 2. Note that 46 μg protein from ATPase peak displays no absorption in the actin region; actin contamination is estimated as less than 1% (cf. Fig. 2 b).

Light Chains

On 15% gels using this buffer system, Physarum light chain (PLC) 2 runs just above hemoglobin; thus, it can be assigned a molecular weight of approximately 17,000 daltons. PLC 1 runs with the second chain of myosin and below chymotrypsinogen (25,700) and can be assigned a molecular weight of approximately 20,000–21,000 daltons.

Examination of the areas under densitometry tracings made of several peak ATPase fractions (cf. Fig. 2 b) shows relative ratios of 16.5:2.7:1 for the heavy and two light chains present. It was previously reported (10) that Physarum myosin heavy chains have on SDS gels on approximate molecular weight of 250,000 ± 10%, considerably greater than that of other heavy myosins. Band splitting of Physarum myosin when coelectrophoresed with muscle myosin has been confirmed (M. R. Adelman, personal communication) and also occurs when Physarum myosin is coelectrophoresed with platelet myosin (R. S. Adelstein, personal communication). The initial estimate of 250,000 is probably somewhat too high. The uncertainty at these high molecular weights is at least 10%. Over a range of 225,000-250,000, however, the molar ratios that would best account for the densities observed here are 1:2:1 for the heavy chain and the two light chains.

Actin Activation

Table II and Fig. 3 demonstrate that the purified Physarum myosin was activated up to tenfold by muscle actin, although four- to fivefold activation (22–24°C) was more often found. Activation increased with increasing actin concentration and leveled off at high actin concentrations (cf. 22). Activation was labile; it was lost when the actin sample was stored as a pellet for 4-5 days, or if the myosin was aged for 1–2 days.

In some preparations (Fig. 3), 0.1 mM calcium salts, which did not significantly alter the actin activation of rabbit muscle heavy meromyosin, in an identical assay (94% of EGTA values), inhibited the actin activation of Physarum myosin some 40%. Hatano and Tazawa (1) found that calcium inhibited the rate of Physarum actomyosin super-
TABLE II

Activation of ATPase of Physarum Myosin by Muscle Actin

| Myosin | Actin | Minutes incubated | Myosin | μmol P/ min/mg |
|--------|-------|-------------------|--------|----------------|
| ml     | ml    |                   |        |                |
| 0.1    | —     | 15                | 0.000  |                |
| 0.1    | —     | 30                | 0.006  |                |
| 0.1    | 0.1   | 15                | 0.066  |                |
| 0.1    | 0.1   | 30                | 0.066  |                |
| 0.1    | 0.1   | 30                | 0.060  |                |
| 0.1    | 0.2   | 15                | 0.095  |                |
| 0.1    | 0.2   | 30                | 0.097  |                |

Conditions: 0.03 M KCl, 0.02 M imidazole, 0.1 mM DTT, pH 7, 24°C. Myosin: peak fraction from 90 cm KI column (see gels in Fig. 2 a), 0.2 mg/ml; Actin from rabbit muscle, approximately 2 mg/ml.* EGTA concentration, 0.75 mM.

* When high actin concentrations are used a small amount of inorganic phosphate is sometimes released in the actin blanks, some 3–5% of the activated values; this has been subtracted.

**Figure 3** Activation of low ionic strength Mg**2+**-ATPase of Physarum myosin by rabbit striated muscle actin. Conditions as in the text. Physarum myosin 0.014 mg/ml.

**Discussion**

All of the properties of Physarum myosin reported here—the unequivocal demonstration of at least two light chains, the severalfold activation of the Mg-ATPase by muscle actin, and the packing of the myosin into different types of filament—serve to emphasize the strong similarity of the myosin effect and to separate effects of calcium on myosin aggregation from other modes of action.

**Filament Formation**

After dialysis of myosin peak fractions against 50 mM KCl, 5 mM imidazole, 1 mM EGTA pH 7, no increase in absorption (650 nm) was detected and no organized filament structures, at least none greater than 0.2 μm in length, were observed by electron microscopy (Fig. 4), while if the same dialysate contained 5 mM MgCl₂ with either 1 mM EGTA (Mg/EGTA) or 0.1 mM CaCl₂ (Mg/Ca) many filaments were observed ranging in size up to 2.5 μm in length (Figs. 5, 8, 9, 10–12, and 13). As these were different from the 0.5 μm long bipolar filaments previously reported (10), the myosin supernatant fraction (Table I a) was reexamined in detail. In agreement with our previous findings, the supernatant fraction formed 0.5 μm compact bipolar filaments in Mg/Ca and loose aggregates in Mg/EGTA (Figs. 6 and 7). When Ca was added directly, an increase in absorbance at 650 nm was always detected at very low (pCa 5) calcium levels, and at 10⁻⁶ M Ca definite precipitates formed containing 70–80% of the CaATPase. The precipitates were aggregated 0.4–0.5 μm bipolar filaments with bulky ends as previously reported (10).

The differences in aggregative properties of the highly purified myosin, “KI myosin”, and the crude myosin supernatant are compared in Table III. Both myosin fractions were soluble in 0.05 M KCl (as defined by centrifugation) and aggregated with divalent ions. They differed in (a) length and packing and (b) magnitude of effects of divalent ions. KI myosin formed longer filaments, some clearly bipolar, others with no bipolarity evident, with a 5–6-nm periodicity (Figs. 10–12 and 13) and, often, a cleft or division running axially (Figs. 10 and 13). The crude supernatant myosin (and also “KI myosin” fractions made directly from actomyosin, not MEAM) formed 0.5–μm bipolar filaments with prominent bulky ends and no fine periodicity.
For Figs. 4-13 the line is 1 μm.

**FIGURES 4 and 5** Effect of magnesium salt in the absence of calcium salts on filament formation by *Physarum* myosin. Fig. 4 Pooled peak fractions of myosin purified as described in the text and dialysed for several hours against ten volumes of 50 mM KCl, 5 mM imidazole, 0.1 mM dithiothreitol (LSB) and 1.0 mM EGTA (pH 7). × 34,000. Fig. 5 Another sample of the same preparation dialysed against the same KCl solution but with 5 mM MgCl₂, 1.2 mM EGTA (pH 7). × 34,000.

from this ameboid-like plasmodium with muscle myosin, as well as with myosin from platelets and fibroblast cells (11, 12). Because of its solubility at low ionic strength, *Physarum* myosin would be most similar to smooth muscle myosin, except that very low or no actin activation has been found with the latter (16). This is probably a minor difference due to problems similar to those encountered with the *Physarum* system—namely, inadequate purification, dependence on actin-myosin ratio, and sensitivity to sulfhydryl oxidation. The present purification method may prove useful for smooth muscle and for other cell types where actin is readily extracted with myosin.
Subunits

Although we and others have found that the heavy chain of Physarum myosin consistently migrated more slowly than platelet or skeletal muscle myosin, when the two were coelectrophoresed on SDS gels (10; M. R. Adelman, personal communication; R. S. Adelstein, personal communication; R. S. Adelstein, personal communication).

FIGURES 6-9 Comparison of the type of aggregates formed by the crude myosin (supernatant fraction) and by myosin purified as described in the text. Fig. 6. Supernatant myosin dialysed against LSB with 5 mM MgCl₂, × 100,000. Fig. 7. Similar preparation 30 min after bringing the calcium (Cl₂) concentration to 0.5 mM, × 80,000. Figs. 8 and 9 Examples of the long filaments formed by dialysis of the myosin purified as described in the text against LSB with 5 mM MgCl₂, 1 mM EGTA (Fig. 3) or 5 mM MgCl₂, 0.1 mM CaCl₂ (Fig. 4) both × 60,000.
FIGURES 10-12 Another set of long filaments. The purified myosin (K1-myosin) dialysed against LSB with 5 mM MgCl₂, 0.1 mM CaCl₂. Fig. 10. × 46,000. The arrows indicate areas where axial splitting is very clear. Comparison of positively stained filaments from myosin purified as described in the text and dialysed against: Fig. 11, LSB with 5 mM MgCl₂, 0.1 mM CaCl₂. × 30,000. Filaments appear identical and both display prominent fringed areas with no apparent bare central zone.
sonal communication), it is clear from the present results that the apparent heavier subunit weight of Physarum myosin is not due to absence of light chains. Like vertebrate smooth muscle myosin (23), fibroblast and platelet myosins (12, 24, 25), Physarum myosin shows only two size classes of light chains. Our present data are best fitted by a molar ratio of the heavy chain to the light chains of 1:2:1. This would imply, since there must be two heavy chains to result in the only available molecular weight estimate of 468,000 (14), a model in which each heavy chain is associated with three light chains. Even at lower estimates of subunit weights, the total exceeds the estimated molecular

![Figure 13](image)

**Figure 13** Enlargement of Fig. 10. x 180,000. Arrows point to loose twisted array (curved arrow) and to linear periodicities (straight arrow).
TABLE III
Effects of Solvent Conditions on the Aggregation of Two Myosin Fractions from Physarum (see Figs. 4-13)

| Solvent conditions* | Myosin supernatant | Myosin after KI treatment |
|---------------------|--------------------|--------------------------|
| 1 mM EGTA           | Soluble (10 K 30°) no filaments | Soluble (10 K 30°) no filaments > 0.2 μm |
| Mg 5–10 mM  
EGTA 1 mM | Loose aggregates | 1 to 2.5 μm filaments |
| Mg 5–10 mM  
Ca²⁺ 0.1 mM | bipolar 0.5-μm filaments with bulky heads; half-filaments common; rapid precipitation of ATPase by aggregates showing head-to-head attachments | 1 to 2.5-μm filaments; no precipitation; fine periodicity of 50-60 A |

* All contain 0.05 M KCl and 5 mM imidazole.

weight by a sizable margin. Therefore a molecular model for this myosin is premature. The definitive point is that two size classes of light chains are found in *Physarum* myosin. One would expect from this finding that other high molecular weight myosins such as are present in *Naegleria* (5), *Dictyostelium* (7), leukocytes (6), and brain (8) will also be found to possess two size classes of light chains.

**Actin Activation**

The dependence of activation on actin concentration is qualitatively similar to that for heavy meromyosin (22) and for muscle myosin during superprecipitation (24). This implies the existence of a complex of actin and myosin which determines the ATPase activity at low ionic strength. However, quantitatively tenfold higher absolute levels of actin are necessary for the plateau with *Physarum* myosin as compared to muscle myosin (24), and the final level of specific activity is lower by a factor of 2. An inhibition at an intermediate level of actin as has been reported for *Acanthamoeba* myosin (26) was not found and no cofactor was required. We did not find any preparations that showed calcium activation; rather, calcium inhibition often occurred.

Calcium activation does not, therefore, reside on *Physarum* myosin still possessed of two light chains.

Only twofold activation (maximum) is found with crude myosin (supernatant fractions, Table I a, step 6). Since a 5% actin contamination results in a 50% molar contamination of myosin with actin, it may be that residual partly denatured actin contaminating crude myosin fractions could result in a masking of activation by added native actin. In agreement with this is the fact that when shorter KI columns were run, resulting in cross-contamination of myosin peaks with actin, only twofold activation by added actin was found.

**Filament Formation**

(Compare Table III.) Highly purified *Physarum* myosin forms filaments more readily than crude myosin, and no longer shows an effect of calcium ions on the aggregation. Magnesium ions at physiological levels stimulate filament formation. The filaments from highly purified myosin are much longer than those from that form from crudier myosin preparations. While filaments from crude myosin form networks in which the aggregation of bulky heads of bipolar filaments is prominent, purified myosin filaments do not.

At present the most likely explanation for the differences is that less pure myosin retains material attached to the head portions of the molecule which sterically impede elongation, and which result in network formation by aggregating head-to-head under the influence of calcium ions. A good candidate is partly denatured actin (see above).

Crude (supernatant) myosin might well be expected to retain traces of actin at the monomer-dimer level: *Physarum* actin filaments appear in a range of sizes (2) so that no definite cut off would be expected; dimers or monomers of partly dena-
tured actin would not cross-link myosin enough to cause precipitation, but would cause the characteristic bulky head regions observed (Figs. 6–9). Such actin (5% contamination) could persist through gel filtration (10). Other myosins have been found to form very long filaments when highly purified (27, 22). In the case of filaments from gizzard myosin, there are similarities with those of Physarum myosin: projections in opposing directions on either side of some filaments (Fig. 13). Some filaments from Physarum seem to have clearcut central bare zones (Figs. 10–12, central pair), while still others display twisted arrangements (Fig. 13, curved arrows) or linear arrays (Fig. 13, straight arrows), suggesting that different types of packing occur.

**In Vivo**

In the absence of data on the free divalent ion content of the cytoplasm of Physarum, one may speculate that it is similar to that of other cells and that the ionic conditions in vivo favor the aggregation of myosin into thick filaments, despite its solubility at 50 mM KCl (13, 14, 15). Wohlfarth-Bottermann and his colleagues have examined Physarum extensively with various fixatives and concluded recently (28) that thick (myosin) filaments are seen in sections from normally streaming plasmodia only after slowly acting fixatives were used, resulting in "convulsive contraction." In a detailed study using glutaraldehyde fixation of Physarum, Rhea (29) could only distinguish 50–60 A filaments in either plasmodia or microplasmodia, while in glycerinated samples of starved microplasmodia Kessler (30) recently described thick filaments. The question now is, since highly purified Physarum myosin forms long filaments in vitro under ionic conditions close to those in vivo—why are special treatments necessary to demonstrate thick filaments in fixed material? There seem to be three possibilities. First, since problems of favorable alignment and optimal fixation exist, and in view of the similar history of thick filament preservation in the case of smooth muscles, only recently resolved (31), a renewed quantitative search for thick filaments in intact Physarum is desirable. The "oligomeric myosin" sliding filament (28, 10) seemed attractive when less pure myosin formed in vitro filaments only at unphysiological levels of calcium. A reassessment is now in order. The oligomers may be large enough to be considered polymers. Second, it is possible that changes in free divalent ion levels can affect the aggregation of Physarum myosin in vivo, as originally proposed for smooth muscle by Schoenberg (32). The third major possibility would be the presence of other, unknown factors that affect myosin aggregation in vivo in Physarum and in other organisms.

**APPENDIX**

**Nomenclature**

It is clear that Physarum "myosin B" (1) or actomyosin (1, 2, 14) is composed of at least myosin and actin. Additional components such as tropomyosin (cf. Fig. 2 a) may also be present. The older but still widely used term "myxomyosin" for Physarum actomyosin (33) retains the connotation of being a single protein. In fact myxomyosin was probably made up largely of actin with some attached, partly obscured myosin (cf. 1, 2). Therefore the special term myxomyosin should now be dropped in favor of simply Physarum myosin.

Myosins similar to muscle myosins in size have been found and characterized in several eukaryotic cell types (11, 12, 25). It seems clear and informative to characterize each myosin prefixed by the name of the cell, e.g., fibroblast myosin, platelet myosin, brain myosin. This rational nomenclature has already been applied to several other cell types (viz., 6, 11, 12, 25), and should be applied also to Physarum as some authors have done (1, 13) since myosin-like proteins are widely distributed.

It is a pleasure to acknowledge the excellent technical assistance of Mr. Andrew Stern and Ms. Melanie Plaut, as well as that of Ms. Joanna Sloane with cultures. Much of the experimental work reported here was carried out in the Department of Biology, Haverford College. This investigation was supported by Grant no. AM-17492 and in part by HL15,835 to the Pennsylvania Muscle Institute from the National Institutes of Health. The major results have been presented (34).

A rapid potassium iodide method for the purification of myosin from platelets and other sources has been independently reported by Pollard et al. (25).

Received for publication 5 November 1973, and in revised form 1 February 1974.

**Note Added in Proof:** H. Hinssen and J. D'Halse report (J. Cell Sci., in press) on similar long filaments from Physarum myosin by a different extraction procedure.

**REFERENCES**

1. **Hatano, S., and M. Tazawa. 1968.** Biochim. Biophys. Acta. 154:507.

---

**64 THE JOURNAL OF CELL BIOLOGY • VOLUME 62, 1974**
1. Nachmias, V. T., H. E. Huxley, and D. Kessler. 1970. J. Mol. Biol. 50:83.
2. Nachmias, V. T., H. E. Huxley, and D. Kessler. 1970. J. Mol. Biol. 50:83.
3. Bettex-Galland, M., and E. F. Lüscher. 1961. Biochim. Biophys. Acta. 49:536.
4. Bettex-Galland, M., and E. F. Lüscher. 1965. Adv. Protein Chem. 20:1.
5. Lastovica, A. J., and A. D. Dingle. 1971. Exp. Cell Res. 66:337.
6. Shibata, N., N. Tatsumi, K. Tanaka, Y. Okamura, and N. Senda. 1972. Biochim. Biophys. Acta. 256:565.
7. Woolley, D. E. 1970. J. Cell Physiol. 76:185.
8. Pusztin, S., and S. Berli. 1972. Biochim. Biophys. Acta. 256:695.
9. Weber, A. 1956. Biochim. Biophys. Acta. 19:345.
10. Nachmias, V. T. 1973. Cold Spring Harbor Symp. Quant. Biol. 37:607.
11. Adelstein, R. S., T. D. Pollard, and W. M. Kuehl. 1971. Proc. Natl. Acad. Sci. U. S. A. 68:2703.
12. Adelstein, R. S., and M. A. Conti. 1973. Cold Spring Harbor Symp. Quant. Biol. 37:599.
13. Hatano, S., and J. Ohnuma. 1970. Biochim. Biophys. Acta. 205:110.
14. Nachmias, V. T. 1972. J. Cell Biol. 52:548.
15. Adelman, M. R., and E. W. Taylor. 1969. Biochemistry. 8:4964.
16. Hamoir, G. 1973. Phil Trans. Roy. Soc. London. B 265:169.
17. Pusztin, S., S. Berli, E. Pusztin, and D. D. Clarke. 1968. Science (Wash. D. C.). 161:170.
18. Straub, F. B. 1942. Stud. Inst. Med. Chem. Szeged. 2:3.
19. Szent-Györgyi, A. G. 1951. Arch. Biochem. Biophys. 31:97.
20. Spudich, J. A., and S. Watt. 1971. J. Biol. Chem. 246:4866.
21. Laemmli, U. K. 1970. Nature (Lond.). 227:680.
22. Mios, C. 1973. Cold Spring Harbor Symp. Quant. Biol. 37:137.
23. Kendrick-Jones, J. 1973. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 265:183.
24. Maruyama, K., and J. Gergely. 1962. J. Biol. Chem. 237:1095.
25. Pollard, T. D., S. Thomas, and T. P. Stossel. 1973. J. Cell Biol. 59(2, Pt. 2):268 a.
26. Pollard, T. D., and E. D. Korn. 1973. Cold Spring Harbor Symp. Quant. Biol. 37:573.
27. Sobieszek, A., and J. V. Small. 1973. Cold Spring Harbor Symp. Quant. Biol. 37:109.
28. Komnics, H., and K. E. Wohlforth-Bottermann. 1973. Int. Rev. Cytol. 34:169.
29. Rhea, R. P. 1966. J. Ultrastruct. Res. 15:349.
30. Kessler, D. 1972. J. Mechanochem. Cell Motility. 1:125.
31. Somlyo, A. P., C. E. Devine, A. V. Somlyo, and R. V. Rice. 1973. Philos. Trans. R. Soc. Lond. Ser. Biol. Sci. 265:223.
32. Shoenberg, C. F. 1969. Tissue Cell. 1:83.
33. Ts'o, P. O. P., L. Eggman, and J. Vinograd. 1956. J. Gen. Physiol. 39:801.
34. Nachmias, V. T., and A. Stern. 1973. J. Cell Biol. 59(2, Pt. 2):244 a.

V. T. Nachmias  Physarum Myosin Purified by Potassium Iodide Procedure  65