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Filament Formation by Purified *Physarum* Myosin

(slime mold/actomyosin/gel filtration/electron microscopy/Ca^{++})

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ABSTRACT ***Physarum*** **myosin can be separated from actomyosin by ultracentrifugation, and purified by gel filtration. Unlike actomyosin, myosin is soluble in 0.05 M KCl in the pH range of 6–7. However, in the absence of actin, the slime mold myosin can be precipitated in 0.05 M KCl by the addition of millimolar concentrations of $CaCl_2$. The precipitates consist of aggregated, short bipolar filaments. Magnesium has a similar effect, but results in the precipitation of more loosely packed aggregates.**

The length of the compact filaments is 0.45 μm; thus, predominantly tail-to-tail, but also some head-to-tail, interactions occur under these conditions. Since the size and shape of these thick filaments are close to those seen in fixed and sectioned ameboid cells and in platelets, all of these filaments are probably composed of myosins.

Actomyosin has been prepared from plasmodia of *Physarum polycephalum* by extraction at high ionic strength and alkaline pH, followed by precipitation at low ionic strength (7, 5, 16, 17). A chelating agent must be present in the first step to obtain a precipitate that redissolves; the procedure can then be repeated to yield three- or four-times precipitated actomyosin. The success of the procedure depends upon the fact that the plasmodial *actomyosin* has solubility properties similar to those of muscle actomyosins.

On the other hand, the *Physarum myosin*, which can be separated from the actin component by additional procedures, is strikingly different in its solubility properties from muscle myosins. Unlike, for example, vertebrate striated muscle myosin, which readily forms filaments at 0.1 M KCl with S values in the range of 70–310 (10, 11, 20), *Physarum* myosin remains soluble even in 0.05 M KCl, and no filaments are visible by electron microscopy despite variation of several parameters (15). Myosin purified by two different approaches forms only small soluble aggregates, with S values of 8–15, at low ionic strengths as compared to S values of 6 at high ionic strength (1, 5, 6).

However, the solubility of *Physarum* myosin is not, apparently, due to any marked difference in shape factors as compared to vertebrate muscle myosins. *Physarum* and vertebrate striated muscle myosin behave similarly on an Agarose 4B column (ref 1. and unpublished observations in this laboratory) and in an analytical ultracentrifuge (5, 6, 1). Both *Physarum* myosin-enriched actomyosin upon negative-staining (17) and metal-shadowed *Physarum* myosin (6) show the existence of an extended, or "tail," portion of the molecule that is at least 120 nm long.

Therefore, the fact that the slime mold myosin does not form filaments at low ionic strength may be due to a charge distribution along the extended part of the molecule different from that characteristic of muscle myosins.

In support of this view, we report here that both partially purified *Physarum* myosin and myosin purified by gel filtration are capable of forming short filaments at low ionic strengths, provided that calcium salts are present.

Partially purified *Physarum* myosin was prepared as described (15). For column purification, the supernatant after precipitation of myosin-enriched actomyosin was concentrated with ultrapure sucrose and applied to a Sepharose 4B column that had been equilibrated with 0.5 M KCl–0.05 M imidazole, pH 7–0.1 mM dithiothreitol and was eluted with the same buffer at flow rates of 10–12 ml/hr. All preparative steps were conducted at 5°. Fig. 1 shows a typical result. An initial asymmetric peak containing all of the calcium-activated ATPase activity is followed by 2 or 3 peaks of uncharacterized material, which absorb strongly at 260 nm. The yield in the ATPase peak was 10–20% of the input material. The peak enzymatic activity, assayed according to Adelman and Taylor (1), was 1 μmol P_i/min per mg of protein, about 2.5 times the activity of the average input material. The ratio of absorbance at 280 nm to that at 260 nm (corrected for light scattering) was 1.6–1.7, as compared to 1.3–1.5 for the input. Plate 1(*a*) shows the result when the input to such a column and the peak fraction were compared on sodium dodecyl sulfate–polyacrylamide gels (21). The number of heavy bands is reduced to one. Some low molec-

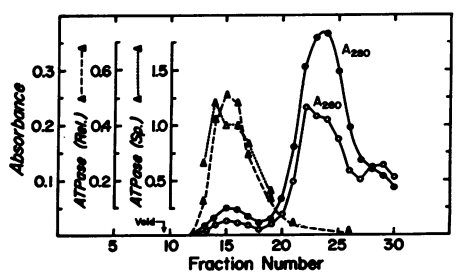

FIG. 1. Gel filtration of *Physarum* myosin. 10 mg of partially purified *Physarum* myosin in 2 ml was applied to a 1.8 × 30 cm Sepharose 48 column, which was equilibrated with, and eluted with, 0.5 M KCl–0.05 M imidazole, pH 7–0.1 mM dithiothreitol. Flow rate was 10 ml/hr. 2.2-ml Fractions were collected, and absorbance at 280 and 260 nm was estimated in a Zeiss PMQ II spectrophotometer. Calcium-activated ATPase was measured by a semi-micro version (15) of the assay conditions of Adelman and Taylor (1).

ular weight material migrates with the tracking dye; however, no conclusions concerning light bands can be made. Electron microscopy of peak fractions showed no actin contamination [see Plate 1(*b*)]. This finding is significant since, if myosin-enriched actomyosin (17) is run on Sepharose 4B columns under dissociating conditions, some actin migrates with the myosin and can be identified by electron microscopy, even when it is not visible as a band on polyacrylamide gels.

Aliquots of the peak fractions from Sepharose columns were dialyzed against 9 volumes of water, or 1, 5, or 10 mM calcium chloride, or 5 mM magnesium chloride. Experiments were run on peak fractions from several different columns, developed at either room temperature or 5°. The results were the same. No precipitation occurred when the ionic strength was simply lowered by dialysis to 0.05 M KCl, and only globular material was visible by electron microscopy. The results were similar to those shown in Plate 1(*b*). Precipitation occurred, however, in all the samples dialyzed against divalent ions within a few hours. The precipitates were dispersed with fine pipets, and drops were applied to filmed grids and examined (16) by the negative contrast technique. The precipitates consisted of masses of short aggregated filaments.

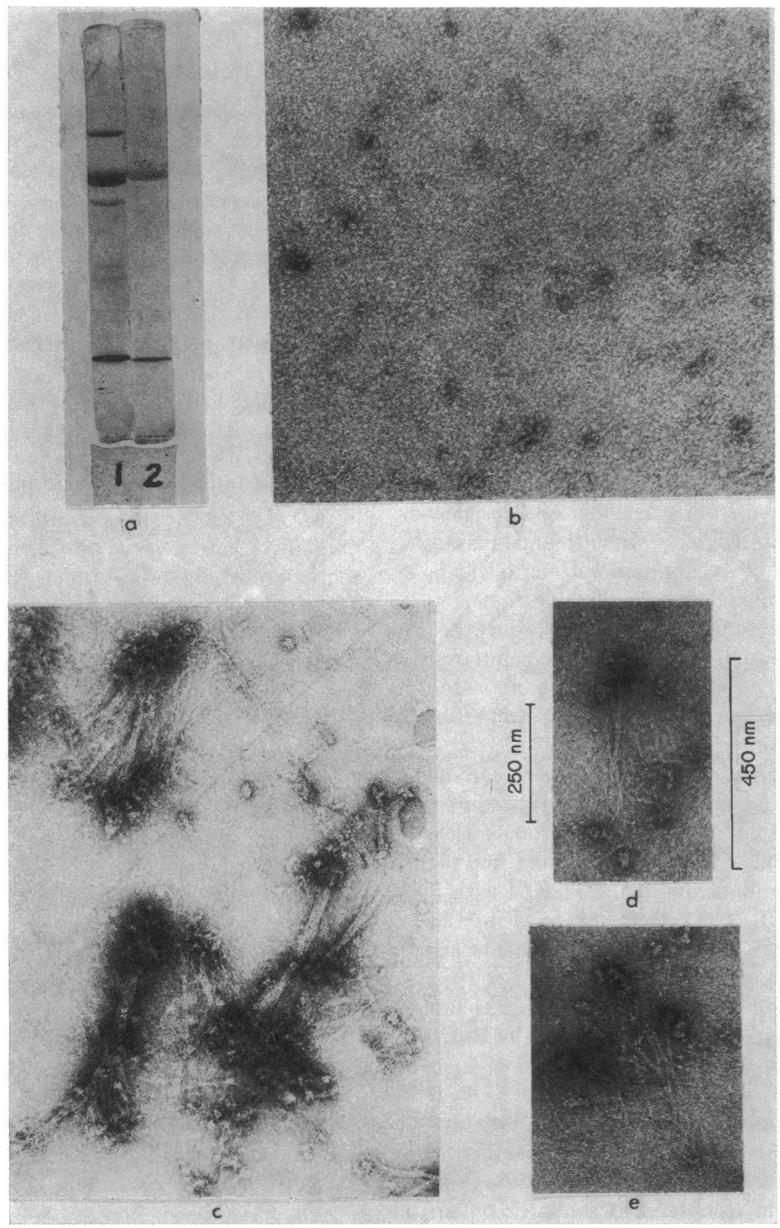

PLATE 1. (*a*). The input to a column as in Fig. 1 is shown on the *left* (*1*) and the peak fraction on the *right* (*2*) as run on 5% polyacrylamide gels in the presence of sodium dodecyl sulfate–2-mercaptoethanol, according to a slight modification of the procedure of Weber and Osborne (21).

Plate 1 (*b–e*) and Plate 2 are examples of negatively stained protein fractions. Magnifications are all ×80,000. (H: tach: HUIIA microscope, 50 kv, 10–15 μA, 50-μm objective aperature).

(*b*). Peak fraction from Sepharose 4B column after dialysis against 0.05 M KCl. No significant aggregates are seen.

(*c*)–(*e*). Examples of aggregates formed when peak fractions from Sepharose columns are dialyzed against 9 volumes of 5 mM $MgCl_2$. Final KCl concentration: 0.05 M; final pH: 7. Note loose structure of the filaments.

In different preparations the packing of individual filaments varied from loose to compact. Compact filaments were found in calcium-treated samples and looser packing was observed in magnesium-treated samples [compare Plate 1(*c–e*) with Plate 2(*a–c*)]. Individual well-formed filaments were 0.45 μm long, with diameters up to 25 nm. All the compact filaments [Plate 2(*e*)] showed distinctive bipolarity, with terminal globular masses separated by a bare central region about 0.2 μm long. Filaments longer than this were not observed, but head-to-head aggregation as in Plate 2(*a*) was frequent.

Although the bulk of the experiments were done with column-purified myosin, filaments could also be formed from partially purified myosin [Plate 2(*b*)]. Therefore, purification is not a requirement for filament formation. Filament formation was reversible, in that treatment of a preparation with excess ethylenediaminetetraacetate resulted in dispersion of the structures.

An additional experiment demonstrated the divalent ion requirement for filament formation by *Physarum* myosin very clearly. *Physarum* was grown in semidefined medium (4), with the addition of 50 μCi of [^{14}C]lysine (New England Nuclear; 312 Ci/mol) for 5 days. The washed, labeled slime-mold (lysine incorporation estimated as 3% after 2 days; mean of 7.0×10^3 cpm/ml of Cl_3CCOOH-insoluble fraction of microplasmodial suspension after 5 days) totaling 5.7 g was pooled with 93 g of unlabeled plasmodium, which had been grown on oatmeal in surface culture. The partially purified myosin was prepared and concentrated, and an estimated 4 mg was mixed with 10 mg of muscle myosin (10)

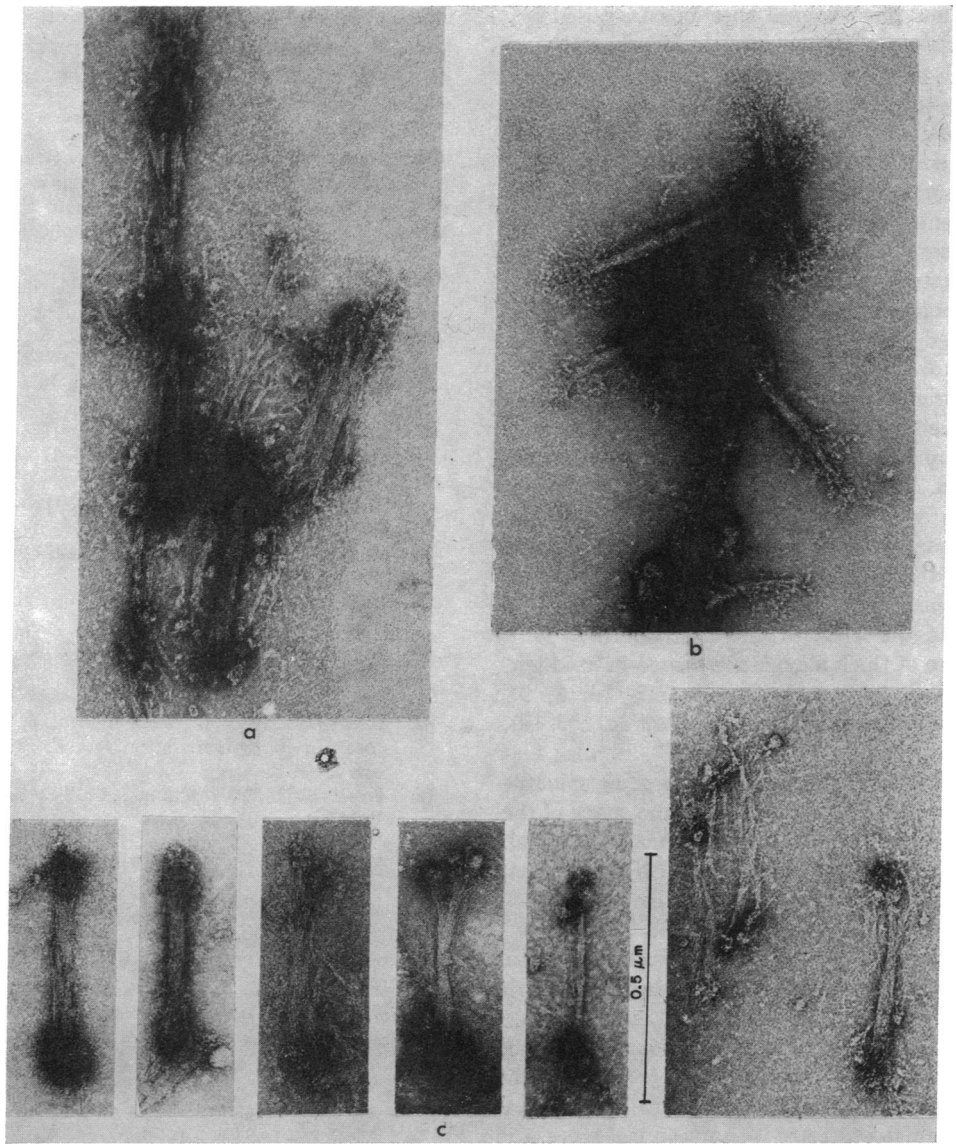

PLATE 2. (*a*). Filaments formed from peak column fractions dialyzed against 9 volumes of 10 mM $CaCl_2$. Note head-to-head aggregations of the bipolar filaments.

(*b*). Input to column (partially purified *Physarum* myosin) dialyzed against 9 volumes of 10 mM $CaCl_2$. The smooth central position of the bipolar filaments is shown very clearly, together with tapering of the extremities, which fray into well-separated globular ends.

(*c*). Examples of individual filaments formed when peak column fractions were dialyzed against 5 or 10 mM $CaCl_2$. The *line* indicates 0.5 μm. Note the uniform lengths of the bare central zone and of the complete filament, and the close packing of filaments formed in the presence of calcium (Plate 2) as compared to magnesium (Plate 1).

in a total volume of 2 ml. The mixture was chromatographed on 4 B Agarose as in Fig. 1, except that 0.5-ml fractions were collected and only the early peak was examined. The peak radioactivity (207 cpm over background) ran two fractions behind the absorbance peak (0.45 at 280 nm).

When peak fractions were diluted to an ionic strength of 0.075, short, thick filaments were observed by negative staining. (Dialysis to the same ionic strength gave long thick filaments.) However, after centrifugation, the radioactivity in the supernatant was still 78% of that in control samples in 0.5 M KCl. Moreover, when the supernatant from these filament preparations was made 10 mM in $CaCl_2$, further turbidity developed; after centrifugation, the radioactivity in the supernatant was indistinguishable from background. Short filaments were observed, but a greater tendency to aggregation was noted.

Several structural and physiological questions are raised by these findings. Perhaps the most accessible is: what is the nature of the small (8–15S) aggregates found in the ultracentrifugal studies of *Physarum* myosin at low salt concentrations? One possibility is that an initial interaction of *Physarum* myosin does not require divalent ions, while further aggregation might have such a requirement, since interactions of different sites on the filaments may be involved. To test whether traces of divalent salt might be required for actomyosin precipitation, which requires tail interactions of the myosin component (15), I prepared *Physarum* actomyosin and examined its ability to reprecipitate in 0.05 M KCl in the presence of EDTA in the range of 10–80 mM. No differences were observed, either in the size of the precipitate or in the absorbance of the supernatants. There does not, then, seem to be any requirement for divalent ions in *actomyosin* precipitation. If the *myosin* interactions that lead to the small aggregates (8–15S) of *Physarum* are the same as those that lead to actomyosin precipitation, then the myosin interactions should also be insensitive to EDTA. It will be interesting to see if this is so.

The dimensions of the thick filaments produced *in vitro* are the same as those of thick filaments reported in fixed and sectioned ameboid cells of various species (13, 14, 3, 9), as well as in an *Amoeba proteus* cytoplasm fraction, (22, 19), and in platelets after certain treatments (23). Similar filaments have also been found in *Physarum* undergoing spherule formation (12). The similarity in dimensions and shape of the filaments from these various cells suggests that they are all composed of myosins. This view is supported by the formation of similar filaments from purified platelet myosin *in vitro*, but without a divalent ion requirement (2).

My results support the general concept that both actin and myosin are necessary for ameboid movement, shuttle streaming, and probably other varieties of cell motility. However, the role of thick filaments as such in cytoplasmic streaming, and also in platelet contractility, is unclear. The formation of large numbers of compact thick filaments has appeared as a terminal aspect of the streaming process in several reports (13, 22, 19). Similarly, *in vitro*, Hinssen (8) found thick filaments, essentially identical to those reported here, in *Physarum* actomyosin that had been treated with relaxing solutions for 18–24 hr. Superprecipitation often occurs under these conditions (Hinssen, personal communication, and unpublished observations in this laboratory). Therefore, it is unclear whether thick filaments may form as a *result* of, or as an *antecedent* to, contraction.

It is intriguing to find that 0.4–0.5 μm appears to be a stable length for the thick filaments in various motile, but not muscle, cells. According to the model put forward by Pepe (18) for the thick filament of striated muscle, a filament 0.44 μm long would have predominantly tail-to-tail interactions, which could give a filament of 354 nm, and two periods (one at either end) of head-to-tail interactions, which could provide another 86 nm in length.

Thus, it seems clear that both tail-to-tail and head-to-tail interactions occur in the filaments I observe. It is interesting to consider that in *Physarum*, divalent ions may be required for only one of these two types of interaction. Should divalent ions be required for tail-to-tail interactions, regulation of contraction could result. This kind of regulation would function in quite a different way than the tropomyosin–troponin system.

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