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Structural States of Dictyostelium Myosin

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Myosin purified from Dictyostelium amoebae has approximately 10% by weight of RNA associated with it, unless specific steps (DEAE cellulose chromatography or RNase digestion) are taken to remove it. This RNA has significant effects on the structural states formed by the myosin at low ionic strength in the presence of Mg^{2+}.

Rapid precipitation of RNA-free myosin by dilution generates bipolar thick filaments (540 nm long, 33 nm thick), often with a bare zone and a 15-nm transverse repeat. Rapid precipitation of myosin with copurified RNA yields linear aggregates of bipolar filaments, showing some lateral association.

Slow precipitation of RNA-free myosin by dialysis yields very long filaments or ribbons (>5 μm, 30–60 nm wide) in which the myosin may be packed diagonally across the filament, similar to the "side-polar" aggregates formed by other nonmuscle myosins and by smooth muscle myosin (Craig R, Megerman J: J Cell Biol 75:990, 1977; Hinssen H, D'Haese J, Small JV, Sobieszek A: J Ultrastruct Res 64:282, 1978). Slow precipitation of myosin with copurified RNA generates linear filaments with repeat intervals of 290 and 650 nm.

Other polyanions were tested for their effects on myosin aggregation. Total RNA and ribosomal RNA from Dictyostelium, when added to RNA-free myosin, also induced the extensive linear aggregation seen with the copurified RNA/myosin complex, although higher concentrations of RNA were required to obtain quantitatively the same effect. DNA and heparin were also effective inducers of linear aggregation, whereas homopolymers of nucleotides and of acidic or basic amino acids were poorly effective.

Key words: myosin, Dictyostelium, RNA, motility, nonmuscle cells

There is no general agreement as to the structural state in which myosin exists in nonmuscle cells. Structures ranging from thick filaments [1] to sheet-like arrays showing periodic immunofluorescence staining [2, 3] have been described. Isolated chick brain myosin forms lateral and head-to-head aggregates of bipolar thick filaments [4]. A similar end-to-end arrangement of myosin from platelets [5] and starfish eggs [6] has been observed

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under certain conditions. Myosin from Amoeba proteus, Physarum polycephalum, blood platelets, and smooth muscle forms elongate filaments with small bare zones near each end [7–9], although the nature of the aggregate formed depends on the rate at which the ionic strength is lowered during precipitation of the myosin aggregates.

A number of workers have noted that myosin isolated from muscle cells is often associated with significant amounts of RNA [10–12]. Baril and co-workers [11] observed heterogeneity of myosin during chromatography on DEAE cellulose, and concluded that this was due to complex formation between polyribonucleotide, myosin, and divalent cations. Pretreatment with RNase or chelating agents yielded a single myosin species, free of ribonucleotide. In the case of chicken muscle myosin, associated RNA is carried through the preparation of LMM, causing significant changes in the structure of paracrystals formed by the light meromyosin (LMM) [12].

It is notable that in the descriptions of unusual aggregated forms of nonmuscle myosin referred to earlier, the preparative methods used generally involve differential precipitation and solubilization, and at no point is RNA likely to be specifically removed from the myosin preparation. An exception to this is the study of Burridge and Bray [4], though their use of DEAE cellulose in the final stages of purification was not invariable and moreover was not specifically identified for those preparations used in the electron microscope analysis.

In the course of experiments with Dictyostelium myosin, we observed that unless specific steps were taken to remove contaminating polynucleotide, the structural state which the myosin adopted varied. We found, moreover, that this effect could be mimicked by certain other polyanions, and that differences in structure could also be obtained by varying the rate at which the ionic strength is lowered. These effects, together with a fine structural analysis of Dictyostelium myosin by negative staining and electron microscopy, are reported below. A preliminary account of part of these observations has been given elsewhere [13].

METHODS

Dictyostelium discoideum was maintained and cultured as described previously [14]. Dictyostelium myosin was prepared as described by Clarke and Spudich [14], with modifications described by Mockrin and Spudich [15]. RNA was extracted from Dictyostelium, fractionated on sucrose gradients, and recovered from the gradient fractions as described by Jacobson [16].

Light scatter measurements were made using a Beckman 35 spectrophotometer, at 300 nm and 21°C, using 1-cm light path cuvettes containing a total volume of 0.5 ml.

For electron microscope analysis of samples, parlodion- and carbon-coated copper grids were used. A drop of sample was placed on a grid, left for 1 min, then rinsed off with six drops of 1% uranyl acetate. The final drop of uranyl acetate was left on the grid for 1 min, then removed with filter paper. After drying, grids were examined at 60 kV using a Philips 300 electron microscope.

Protein was assayed by the method of Bradford [17]. RNA in purified samples was quantitated by absorbance measurements at 260 nm or, in the case of samples containing protein, by measurement of absorbance at 260 nm of supernatants obtained after treatment with 1 M KOH for 2 h at 37°C, followed by precipitation with an equal volume of 10% (w/v) $HClO_4$.

RNase A and DNase I were obtained from Worthington; heparin from Calbiochem;

DNA (salmon sperm), yeast tRNA, polyadenylic acid (poly A), polycytidilic acid (poly C), polyadenylic-cytidilic acid (poly AC), polyaspartic acid, polyglutamic acid, and polylysine from Sigma. Tubulin (chick brain) was a generous gift from Dr. L. Honig, University of California, Berkeley.

RESULTS

Filament Formation by Myosin With and Without RNA

Myosin prepared from Dictyostelium by the procedure of Clarke and Spudich [14] is more than 95% pure, as judged by electrophoretic analysis on acrylamide gels stained for protein. However, Dictyostelium myosin prepared by this method contains a significant amount of RNA. The ratio of absorbances at 280 and 260 nm was found to be 0.5–0.7, and treatment of this myosin for 2 h at 37°C with 1 M KOH or with 20 μg/ml pancreatic RNase yielded acid-soluble nucleotides or oligonucleotides equivalent to 50–150 μg RNA/mg protein. DNase I treatment did not generate significant amounts of acid-soluble nucleotide (<2 μg/mg protein). Precipitation of this RNA-containing myosin preparation at low ionic strength with 5 or 10 mM Mg^{2+} resulted in the formation predominantly of elongate filaments, sometimes branched, apparently composed of bipolar thick filaments aggregated head-to-head, and sometimes side-to-side (Fig. 1).

Chromatography of this myosin on DEAE cellulose, or digestion with RNase and reprecipitation, reduced the RNA content to less than 2 μg RNA/mg protein (the limit of detection by the method of RNA determination used), and the 280/260 nm ratio increased to 1.6–1.8. This RNA-free myosin, on dilution into low-ionic-strength, Mg^{2+}-containing buffer, yielded independent bipolar thick filaments almost exclusively (Fig. 2). More than 95% of the myosin precipitated under these conditions, whether RNA was present or not.

Nature of the RNA Associated With Myosin

The size range of RNA species associated with Dictyostelium myosin is illustrated in Figure 3. RNA molecules ranging from approximately 4S (about 2×10^4 daltons) to 18S (about 10^6 daltons) or more were found, with discrete size classes evident. However, the relative amount of different size classes of RNA in different preparations of myosin varied from one preparation to another. It should be noted that no particular precautions were taken to limit RNase activity during the homogenization of the cells, or during extraction and purification of the myosin.

Specificity of the Interaction of Myosin With RNA

A simple method of analysis of the RNA-myosin interaction was provided by measuring light scattering generated when myosin was precipitated by dilution into buffer containing low salt and Mg^{2+}. With myosin free of RNA, there was a small increase in light scattering as measured by the change in absorbance at 300 nm (Fig. 4); 3 min after dilution more than 95% of the myosin could be sedimented by centrifugation under conditions designed to clear particles 100S or greater in size, which is the approximate sedimentation coefficient of bipolar thick filaments. Electron microscope examination revealed that the preparations contained individual bipolar thick filaments and few, if any, aggregates of these.

Addition of RNA (recovered by high-salt elution of DEAE cellulose used to chromatograph myosin in the final purification step) to RNA-free myosin resulted in a further fourfold to fivefold increase in A_{300} of the myosin suspension (Fig. 4). As judged by elec-

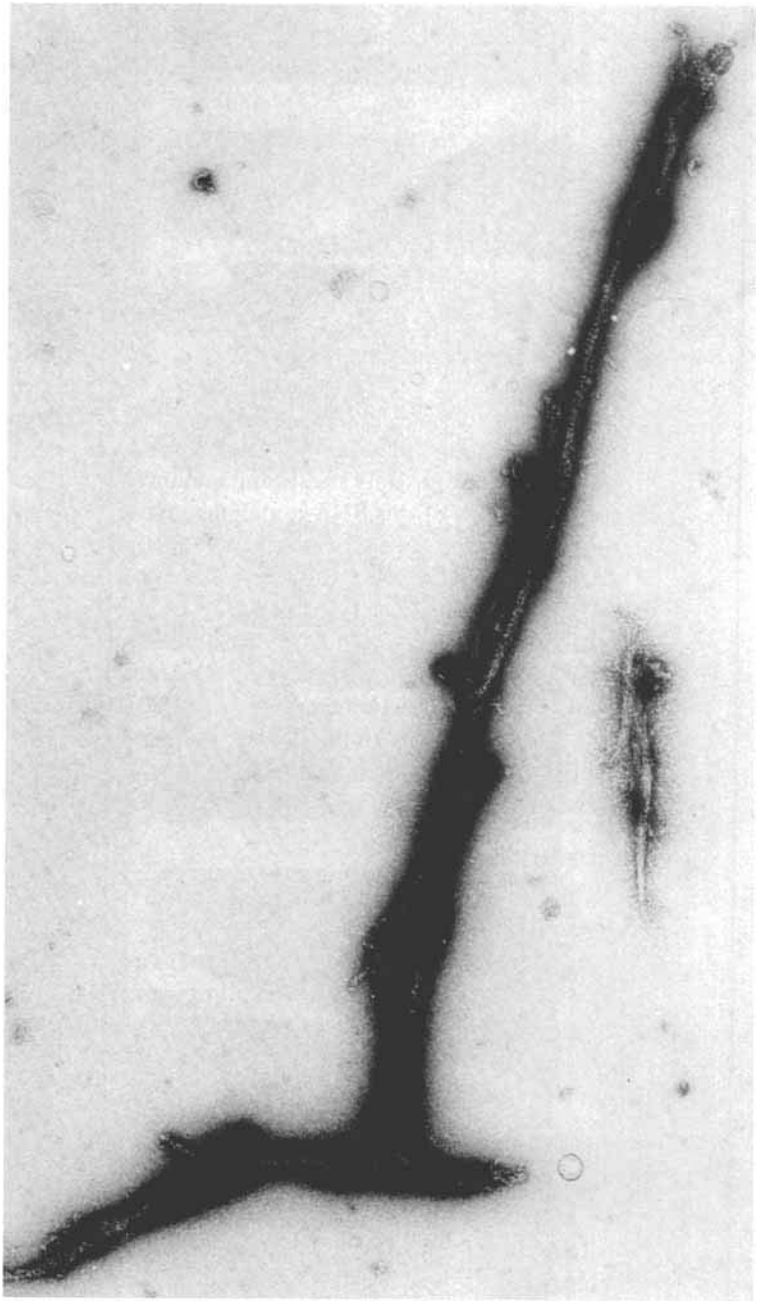

Fig. 1. Electron micrograph of rapidly precipitated Dictyostelium myosin carrying RNA. Dictyostelium myosin, with copurified RNA (85 μg/mg protein), was diluted 40-fold from stock solution (4.4 mg protein/ml in 0.5 M KCl, 10 mM Tris-Cl, 1 mM EDTA, 0.2 mM DTT, pH 7.5) into 10 mM Tris-maleate, 10 mM $MgCl_2$ (pH 6.5) and was incubated at 21°C for 10 min. Samples were stained with uranyl acetate and examined by electron microscopy as described in Methods. × 54,000.

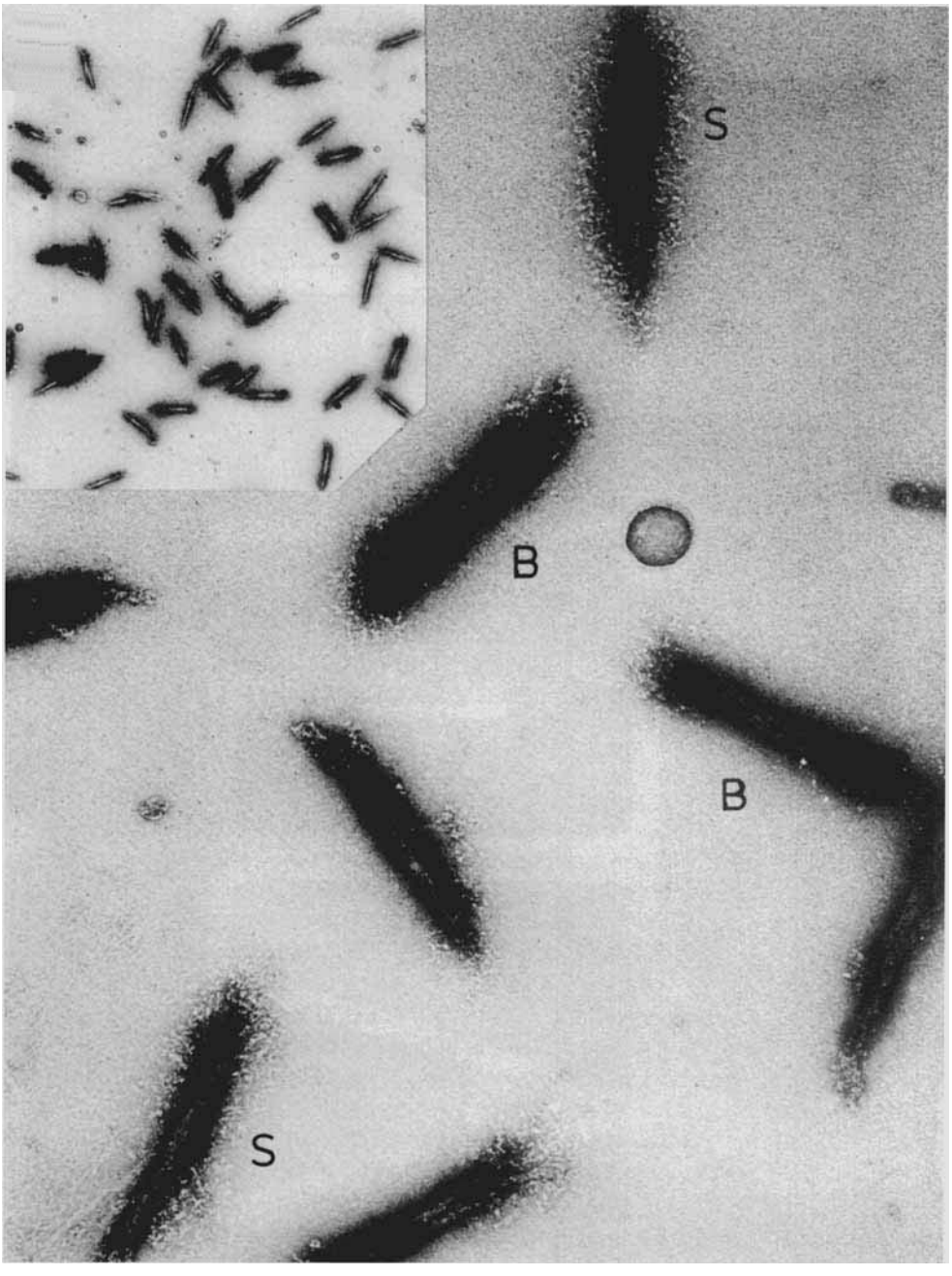

Fig. 2. Electron micrograph of Dictyostelium myosin freed of RNA by RNase A digestion. Myosin (4.2 mg/ml) was incubated with 20 μg RNase A per milliliter in 0.5 M KCl, 10 mM Tris-Cl, 1 mM EDTA (pH 7.5) for 2 h at 30°C. DTT was then added to a final concentration of 0.2 mM, and the myosin was precipitated by dilution 40-fold into 10 mM Tris-maleate, 10 mM $MgCl_2$ (pH 6.5) and incubated at 21°C for 10 min. Samples were stained and examined by electron microscopy as described in Methods. As controls, incubation at 30°C for 2 h in the absence of RNase, or addition of RNase (20 μg/ml) without this incubation, did not inhibit the formation of highly aggregated states such as those seen in Figure 1. Inset is a general view showing split and twisted variants of thick filaments. The enlarged region shows spindle (S) and blunt-ended (B) forms of the filaments. × 89,000; inset × 15,000.

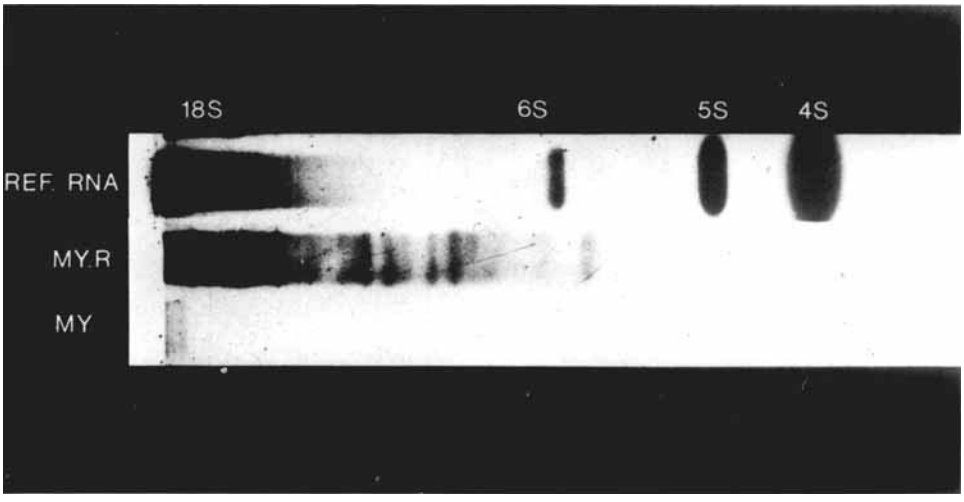

Fig. 3. SDS gels of RNA species in Dictyostelium myosin preparations made by the procedure of Clarke and Spudich [14]. A myosin preparation (20 μg) containing 2.2 μg copurified RNA (MY.R), and 26 μg of myosin freed of RNA by DEAE cellulose chromatography (MY), were electrophoresed on 10% polyacrylamide-SDS gels. The gels were then stained with ethidium bromide (15 μg/ml in 1 mM EDTA) for 20 min and photographed under UV (260 nm) excitation using Polaroid T50 black and white film (6 min exposure, yellow filter). Drosophila RNA (1 μg) containing 4S, 5S, 5.8S, and 18S species was run as standard. Myosin heavy-chain migrates in the gel approximately 10% of the distance of 4S RNA under the conditions used.

tron microscopy, the increase in A_{300} appeared to be a consequence of association of individual thick filaments to form larger aggregates of the type shown in Figure 1. A similar total change in A_{300} was seen when myosin already complexed with copurified RNA was caused to aggregate by dilution into low salt plus Mg^{2+}. In each case with RNA present, the aggregates could be quantitatively cleared by centrifugation as described above. After formation of these aggregates, subsequent addition of salt to the reaction cuvette decreased the A_{300}, ultimately returning to nearly zero at 0.3 M KCl (Fig. 4); neither bipolar thick filaments nor aggregates were then present, as judged by electron microscopy or by centrifugation. Half-reduction in light scatter was achieved at 0.1 M KCl with RNA-free myosin and at 0.07 M KCl in the case of myosin complexed with RNA.

These observations indicate that the increase in A_{300} that results from dilution of myosin into low salt plus Mg^{2+} buffer is due to formation of bipolar thick filaments and to the generation of larger aggregates of them if RNA is present.

The amount of RNA required to cause the formation of large aggregates of myosin, such as seen in Figure 1, can be obtained by titrating the extent of change of A_{300} upon addition of increments of RNA, and determining either the end point or the point of half-maximal effect. The latter has been used to compare the effects of a range of polyanions and polycations (Table I).

Total cellular RNA from Dictyostelium induced half-maximal aggregation when RNA was present to the extent of 150 μg/mg myosin. When total cellular RNA was fractionated on sucrose gradients, the effect was greatest using the high-molecular-weight, or ribosomal, RNA fraction. RNA extracted directly from myosin was also more effective than total RNA as an inducer of aggregation (Table I).

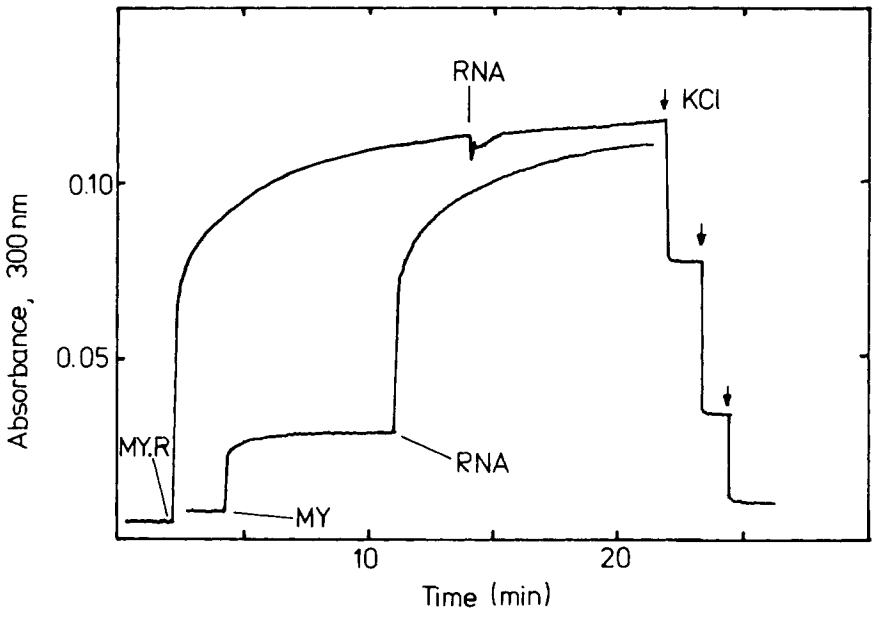

Fig. 4. Light scattering by myosin diluted into low-ionic-strength buffer. Myosin with copurified RNA (MY.R; 85 μg RNA/mg protein) was diluted at time = 2 min from stock solution (4.5 mg protein per milliliter in 0.5 M KCl, 10 mM Tris-Cl, 1 mM EDTA, 0.2 mM DTT, pH 7.5) 20-fold into 10 mM Tris-maleate, 10 mM $MgCl_2$ (pH 6.5) in a spectrophotometer cuvette (0.5 ml final vol). In a separate experiment myosin without RNA (MY) (RNA removed by DEAE cellulose chromatography) was similarly diluted at time = 4 min. Absorbance was recorded continuously at 300 nm, 21°C. At times indicated, 10 μg RNA recovered from myosin by phenol extraction and ethanol precipitation [16] was added. At each of the times indicated by the small arrows, 25 μl of 2 M KCl was added to the diluted MY.R.

High-molecular-weight DNA was the most effective inducer of aggregation tested, and a significant effect was also seen with heparin, a high-molecular-weight sulphated polysaccharide. The aggregates formed, when examined electron microscopically, were similar to those shown in Figure 1. Poly C partially induced aggregation, but yeast tRNA, poly A, polyAC, polyaspartic acid, and polyglutamic acid are polyanions which exhibited little effect. In those cases where polyanions generated aggregates, salt lability was always seen.

RNase A, a cationic protein which induces assembly of tubulin to form microtubules [18, 19], and tubulin itself, were each only weakly effective in causing Dictyostelium myosin to aggregate. The polycation polylysine induced substantial aggregation at low concentration, but the aggregates formed were not labile in 0.3 M KCl. Furthermore, electron microscope analysis revealed amorphous aggregates with none of the apparent lateral or linear structure of the sort seen in Figure 1.

Structural Features of Bipolar Thick Filaments and Elongate Filaments Formed by Dictyostelium Myosin

Bipolar thick filaments. The bipolar thick filaments formed by Dictyostelium RNA-free myosin under conditions of rapid precipitation were similar to those described for nonmuscle cells of animal origin [20] except that they were bigger (Fig. 2). Single filaments had a mean length of 540 ± 12 (SEM, n = 20) and thickness (in the central bare zone) of 33 ± 1 (SEM, n = 20).

TABLE I. Effect of Polyionic Molecules on Dictyostelium Myosin Aggregation

	Amount of polyion required to effect 50% increase in maximum light scatter (μg/mg myosin)
Dictyostelium total RNA	150
rRNA	68
8-12S RNA	>1,000
tRNA	>1,000
myosin RNA	91
DNA (salmon sperm)	45
tRNA (yeast)	510
Heparin	110
RNase	No effect
Tubulin	>1,000
Poly A	>500
Poly C	320
Poly AC	No effect
Poly asp	>500
Poly glu	No effect
Poly lys	>500[a]
ATP	No effect

[a]Myosin (RNA-free) was diluted into 10 mM Tris-maleate, 10 mM $MgCl_2$ (pH 6.5), as described in the legend to Figure 4. Sequential additions of concentrated stock solution of the polyionic compounds listed (also in Tris-maleate, $MgCl_2$) were made and the increment, if any, was recorded and corrected for dilution effects. The amount required to bring about 50% of the light-scatter increment obtained with myosin containing copurified RNA (85 μg/mg myosin protein), diluted in the same way to the same protein concentration, was estimated (see Fig. 4 for extent of light-scatter increase); this myosin did not respond further to addition of RNA (Fig. 4) or of other polyions.

Two apparently different types of bipolar filament were commonly observed in the same preparation. Among the 20 randomly selected filaments measured for length and thickness, 12 were spindle-shaped, apparently with heads projecting from the entire length of the filament, that is, without any obvious bare zone (Figs. 2 and 5). The remaining eight filaments were blunt-ended, without the tapering shape of the first group, and contained a bare zone from which heads were apparently absent (Figs. 2 and 5). Some twisting of the filament structure occurred in nine cases (distributed over both tapered and blunt forms), and three of these showed some type of "splitting" of the filament (Fig. 2, inset). For each 20 filaments examined, approximately one filament up to twice the mean length (but normal thickness) could be seen. These appear not to be simply aggregates of individual filaments, but extended normal filament structures (Fig. 2, inset).

A repeat pattern, with an interval of 14–16 nm, was sometimes apparent in bipolar filaments (Figs. 1 and 5). This repeat interval is commonly observed in X-ray diffraction patterns of muscle, and is interpreted as a measure of the true meridional repeat along the myosin thick filament [21].

Continuous elongate filaments. Precipitation of Dictyostelium RNA-free myosin by dialysis, rather than by rapid dilution, resulted in the formation of filaments which were

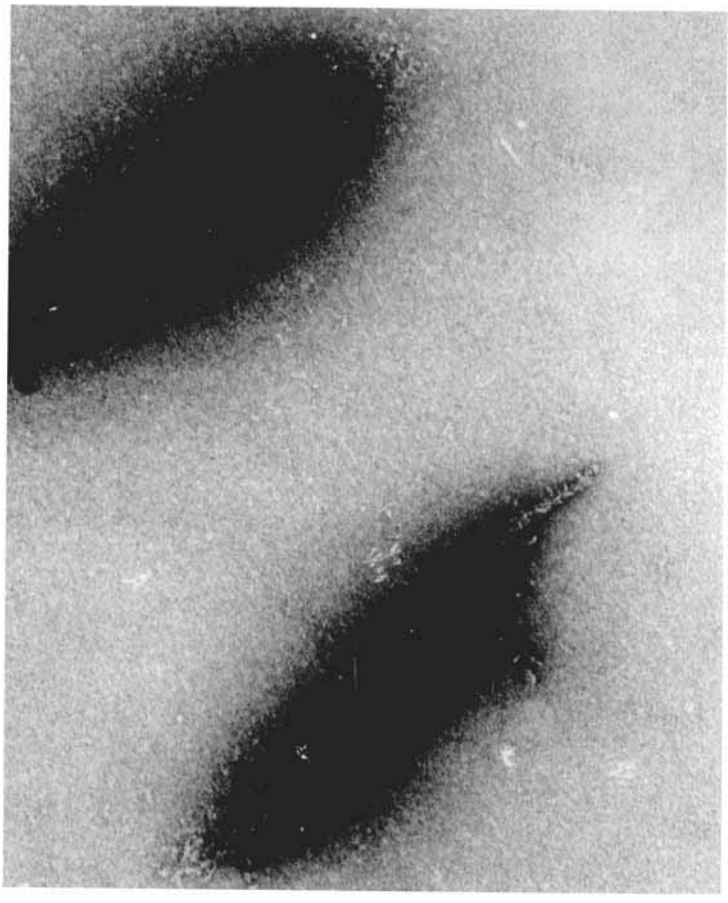

Fig. 5. Electron micrograph of RNA-free myosin illustrating a 15-nm repeat along the longitudinal axis of bipolar thick filaments. Preparation was as described in Figure 2. × 109,000.

continuous for lengths of 5 μm or more (Fig. 6), that is, ten or more times the length of the bipolar thick filaments which form on rapid dilution. There was no evidence, in these RNA-free myosin preparations, of a long interval repeat which might suggest, for example, a linear assembly of bipolar thick filaments that has been described for chick brain [4] and sea urchin myosins [6], and that is seen in the myosin-RNA complex described below.

These elongate filaments may be flattened, like a tape rather than a cylinder, with heads projecting along one or both edges. Where the aggregation of molecules is looser than normal (Fig. 6b), an arrangement of myosin subunits which is diagonally transverse across the main "tape" structure can be discerned. This is similar to the organization of myosin molecules in smooth muscle filaments observed by Craig and Megerman [22].

A somewhat different form of elongate filament was generated when myosin carrying copurified RNA was dialyzed against low salt plus Mg^{2+} buffer (Fig. 7). This form may represent head-to-head aggregation (plus some overlap) of bipolar thick filaments, since in these preparations individual bipolar filaments are sometimes seen, and this type of head-to-head arrangement occurred also when the RNA-containing myosin was precipitated

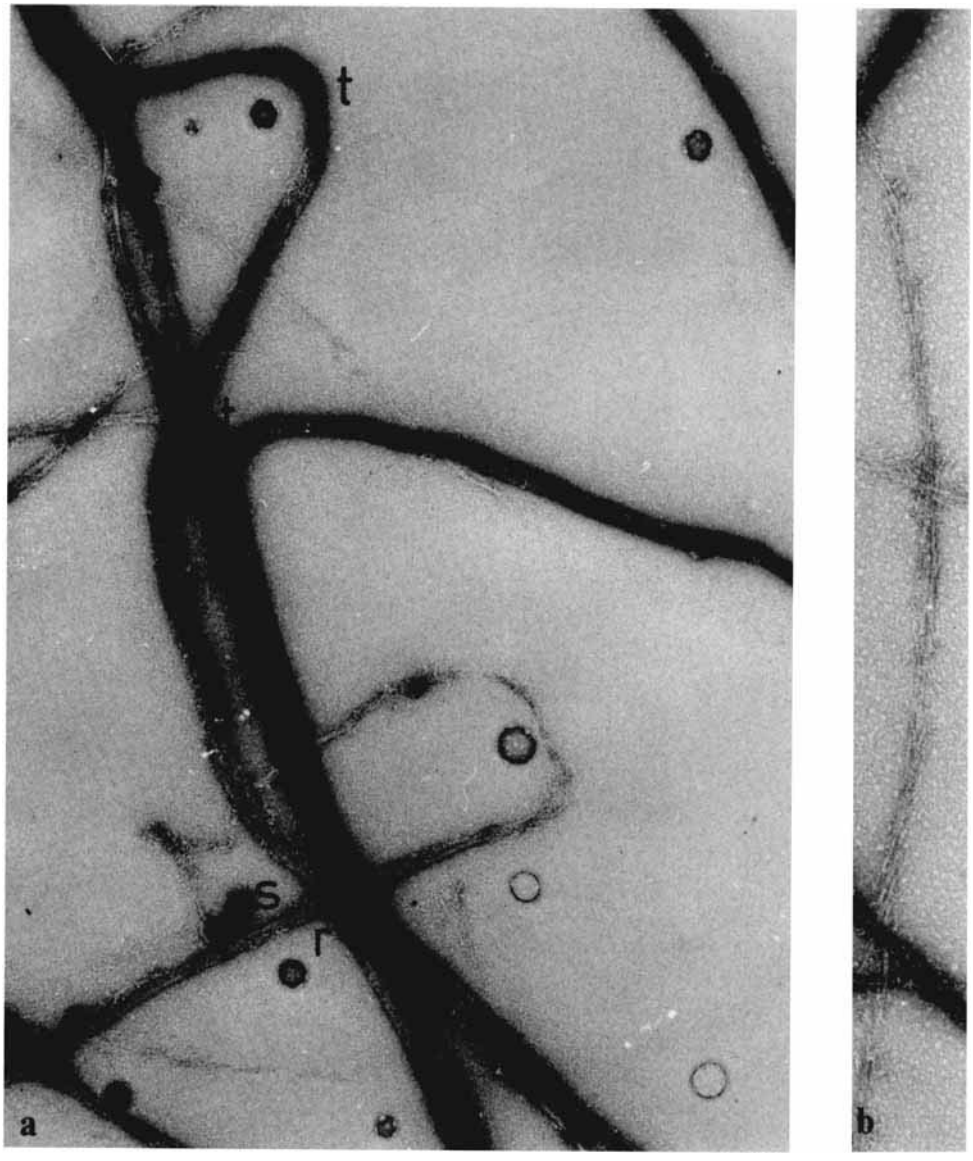

Fig. 6. Electron micrographs of elongated filaments formed from RNA-free myosin. Stock myosin (4.3 mg/ml in 0.5 M KCl, 10 mM Tris-Cl, 1 mM EDTA, 0.2 mM DTT, pH 7.5) was dialyzed for 24 h against 1,000 volumes of 10 mM Tris-maleate, 10 mM $MgCl_2$ (pH 6.5) with continuous stirring. The myosin was then diluted to approximately 0.2 mg/ml with dialysis buffer and prepared for electron microscopy. a: Examples of apparent twisting of the filaments (t), and smooth (s) and rough (r) zones along the filament. b: Loosely aggregated material in a filament, suggesting angular packing of myosin monomers or oligomers. × 53,000 (a); × 44,000 (b).

rapidly (Fig. 1). Some filaments showed a repeat of about 650 nm and others a repeat of about 290 nm (Fig. 7). The 290-nm repeat may represent bipolar thick filaments that are laterally associated and out of register by approximately half of a single bipolar filament length. This type of packing resembles that proposed for uterine myosin by Wachsberger and Pepe [23].

DISCUSSION

Filamentous aggregates or structures which appear to contain myosin have been described in situ in Amoeba proteus [1], cultured animal cells [24], Dictyostelium [25], and intestinal epithelial cells [26]. These instances are exceptional, however, and the failure generally to identify myosin filaments in thin sections, despite its demonstrable presence by fluorescent antibody staining methods [2, 3], has important implications for models describing the location and organization of myosin in nonmuscle cells.

It is interesting that the ~0.5-μm periodicity of the linear aggregates of RNA-containing myosin described here is similar to the periodicity of the myosin immunofluorescence staining pattern seen in stress fibers [2, 3]. It is possible that such linear aggregates of myosin are associated with the actin-containing stress fibers in the cell and that myosin antibodies have preferentially labeled the globular head portion or the tail portion of the myosin molecules (for example, see the longer linear aggregate in Fig. 7).

The possibility that myosin exists only transiently as filaments, and as individual molecules or partially assembled filaments under physiologic conditions, has not been examined experimentally. In general, myosin is more soluble at high ionic strengths. Mabuchi [6] found that half-maximal assembly of bipolar filaments of purified myosin from sea urchin eggs occurred at approximately 0.25 M KCl, which is considerably higher than that (approximately 0.07 M KCl) which he found for skeletal muscle myosin; the ionic strength of the intracellular milieu of the sea urchin egg is thought to be about 0.25. The assembly of Dictyostelium myosin filaments is sensitive to 0.07–0.1 M KCl, when measured by the light scatter assay. Although other ions and components of the cytoplasm undoubtedly influence the critical ionic strength for myosin aggregation and filament assembly, this value is sufficiently near in situ conditions to indicate that in the cell there may be a significant fraction of myosin not present as filaments.

The possibility that RNA, or some other polyanion, may be physiologically important as an effector or template for myosin filament assembly is raised by the observations that RNA copurifies with myosin through most of the purification procedure used and that it has a marked effect on the aggregation of myosin induced by either fast or slow precipitation. This RNA is heterogeneous in size, but cleavage of a more homogeneous species of RNA may have occurred during isolation of the myosin-RNA complex. It is also possible that the RNA that associates with the myosin is truly heterogeneous and that the effects of RNA are quite nonspecific. These effects may be induced by any one of a variety of polyanions that may be abundant in the cell and that can associate tightly with myosin for totally nonphysiologic reasons. A clearer understanding of the possible biologic significance of the phenomenon will require the fractionation and systematic testing of individual RNA species for their effect on myosin.

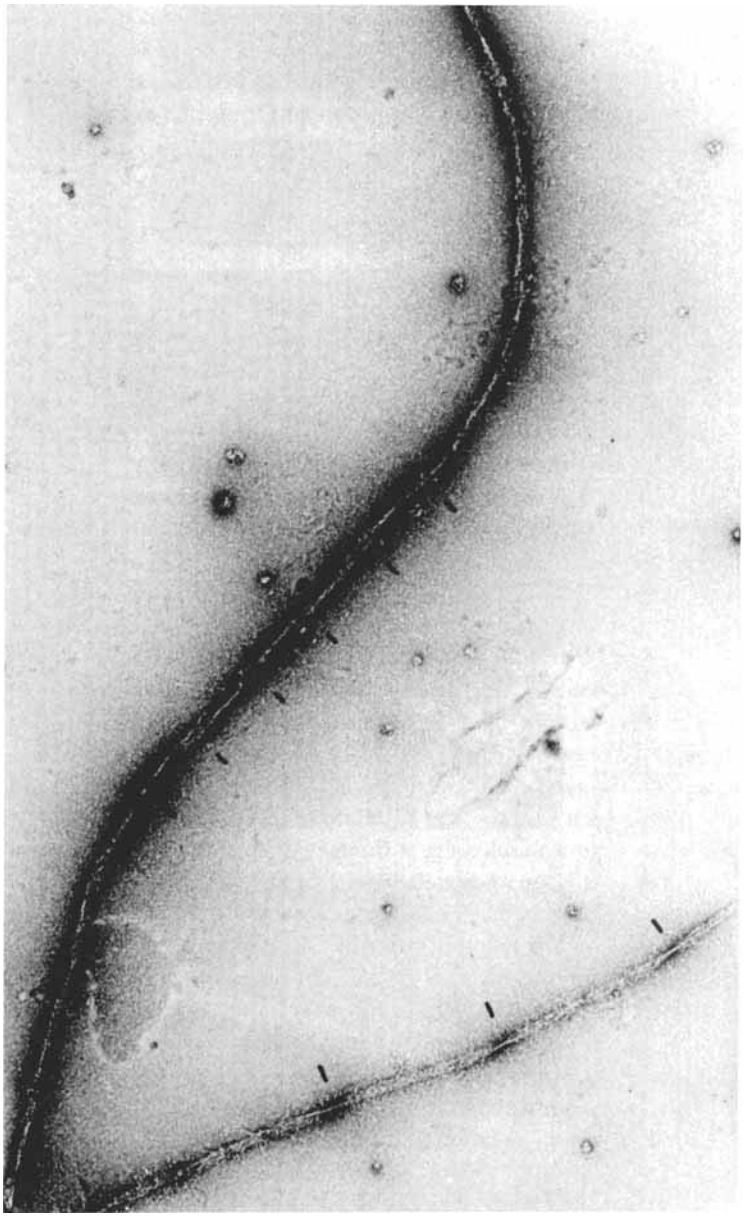

Fig. 7. Electron micrograph of elongated filaments of myosin with complexed RNA. Myosin (110 μg RNA per milligram protein; 4.4 mg protein per milliliter in 0.5 M KCl, 10 mM Tris-Cl, 1 mM EDTA, 0.2 mM DTT, pH 7.5) was dialyzed, diluted, and prepared as described in the legend to Figure 6. Two somewhat different types of elongated filaments are seen, one with a mean repeat distance of 290 nm, the other 650 nm. × 38.000.

Structurally, the formation of bipolar thick filaments on the one hand, and ribbons or continuous filaments on the other, probably reflects alternative capabilities of the myosin monomers to pack to form stable, supramolecular structures. In the bipolar filaments, alternative packing of myosin molecules may be possible, giving rise to spindle-shaped structures without bare zones, or square-ended filaments with prominent bare zones. Within the bipolar form, the presence of cross striations with spacing 15 nm presumably reflects packing of molecules similar to that described for skeletal muscle myosin [21].

The longer ribbons or filaments which form from dialyzed RNA-free myosin may be analogous to the "side-polar" or "antiparallel" filaments proposed for smooth muscle myosin by Craig and Megerman [22].

Alternative assembly states for myosin monomers or oligomers to form bipolar filaments on the one hand, and continuous filaments or tapes on the other, could be important in terms of different contractile functions in which myosin may be involved. The bipolar filament, operating in an array of actin filaments similar to that in the sarcomere of a striated muscle myofibril, may have limited capacity to move actin filaments axially, a consequence of the capacity of each end of the myosin filament to interact only with actin filaments of appropriate polarity [22, 27]. As pointed out by Craig and Megerman [22] and by Hinssen et al [9], sidepolar, elongate myosin filaments, with myosin heads of single polarity along one side of the filament and reverse polarity on the other side, may have a capacity to move actin filaments over longer axial distances and thus have a greater potential for shortening. Dual systems involving combinations of short bipolar myosin filaments and elongate side-polar filaments could be important in providing a diversity of contractile functions in nonmuscle cells.

Until the actual structural states which myosin adopts in nonmuscle cells in situ are known, these proposals can be only speculative. The rate at which filament formation occurs, the concentration of salt and divalent cations, and the presence of certain polyanions are potentially significant in determining the supramolecular state adopted in vivo. The quantitative rarity of myosin as compared with actin in nonmuscle cells makes it difficult to address this question directly, at the electron microscope level. Indeed, it is possible that myosin coexists in a number of supramolecular and soluble states, depending on the particular motile functions expressed at the time. Proteins, other macromolecules, and small molecules may be important regulators in determining the balance between these states.

REFERENCES

1. Holbertson DV, Preston TM: Exp Cell Res 62:473, 1970.
2. Ash JF, Vogt PK, Singer SJ: Proc Natl Acad Sci USA 73:3603, 1976.
3. Fujiwara K, Pollard TD: J Cell Biol 71:848, 1976.
4. Burridge K, Bray D: J Mol Biol 99:1, 1975.
5. Pollard TD, Fujiwara K, Niederman R, Maupin-Szamier P: In Goldman R, Pollard T, Rosenbaum J (eds): "Cell Motility." Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1976, p 689.
6. Mabuchi I: J Mol Biol 100:569, 1976.
7. D'Haese J, Hinssen H: Cell Tissue Res 151:323, 1974.
8. Hinssen H, D'Haese J: J Cell Sci 15:113, 1974.
9. Hinssen H, D'Haese J, Small JV, Sobieszek A: J Ultrastruct Res 64:282, 1978.

10. Mihalyi E, Laki K, Knoller MI: Arch Biochem Biophys 68:130, 1957.
11. Baril EF, Love DS, Herrmann H: J Biol Chem 241:822, 1966.
12. Chowrashi PK, Pepe FA: J Cell Biol 74:136, 1977.
13. Stewart PR, Spudich JA: In Pepe FA (ed): "Motility in Cell Function." New York: Academic Press, 1978.
14. Clarke M, Spudich JA: J Mol Biol 86:209, 1974.
15. Mockrin SC, Spudich JA: Proc Natl Acad Sci USA 73:2321, 1976.
16. Jacobson A: In Last JA (ed): "Eukaryotes at the Subcellular Level." New York: Marcel Dekker, 1976, p 161.
17. Bradford M: Anal Biochem 72:248, 1976.
18. Erickson HP: In Goldman R, Pollard T, Rosenbaum J (eds): "Cell Motility." Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1976, p 1069.
19. Zackroff RV, Rosenfeld AC, Weisenberg RC: J Supramol Struct 5:577, 1976.
20. Pollard TD: J Cell Biol 68:579, 1976.
21. Huxley HE, Brown W: J Mol Biol 30:383, 1967.
22. Craig R, Megerman J: J Cell Biol 75:990, 1977.
23. Wachsberger PR, Pepe FA: J Mol Biol 88:385, 1974.
24. Goldman RD: J Cell Biol 52:246, 1972.
25. Spudich JA, Clarke M: J Supramol Struct 2:150, 1974.
26. Mooseker MS, Tilney LG: J Cell Biol 67:725, 1975.
27. Spudich JA: J Biol Chem 249:6013, 1974.