THE SELF-ASSEMBLY OF SYNTHETIC FILAMENTS OF MYOSIN ISOLATED FROM CHAOS CAROLINENSIS AND AMOEBA PROTEUS

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SUMMARY

Synthetic myosin thick filaments were formed from preparations of electrophoretically homogeneous myosin isolated from Chaos carolinensis and Amoeba proteus when dialysed to physiological ionic strength and pH. Myosin dialysed directly against low ionic strength buffers formed native-like thick filaments in the presence and absence of exogenous divalent cations. The average dimensions of the synthetic filaments grown under these conditions were 455 nm long and 16 nm wide with a distinct bare central zone 174 nm long.

Myosin predialysed against EGTA-EDTA solutions at high ionic strength and then dialysed to low ionic strength formed native-like filaments only in the presence of 1 mM Mg²⁺. 1 mM Ca²⁺ could not be substituted for Mg²⁺ under these conditions to achieve native-like filaments. Filaments grown from predialysed myosin in the absence of Mg²⁺ resembled EGTA-dissociated myosin filaments observed in EGTA-treated cytoplasm and were highly branched, poorly formed filaments lacking a distinct bare central zone. The average dimensions of the filaments grown from predialysed myosin in the absence of Mg²⁺ were 328 nm long, 13 nm wide with a bare central zone 111 nm long.

Under the conditions tested, myosin isolated from these amoebae did not demonstrate a divalent cation requirement for thick filament formation. The results obtained with myosin isolated from the 2 organisms were identical.

INTRODUCTION

The work of many investigators has demonstrated that actin is a highly conserved protein, similar in form and function regardless of its source (Pollard & Weihing, 1974). Myosin, however, is often quite variable in both its enzymic and physical properties (Pollard & Weihing, 1974; Condeelis, 1977). Indeed, these differences may account for much of the variety of motile behaviour observed in nature.

Differences in the physical properties of myosin from different organisms might result in differences in size and stability of the myosin aggregates. How would this affect differences in streaming behaviour? The connexion might be found in the sliding filament hypothesis proposed for muscle contraction (Hanson & Huxley, 1960) and later generalized to cytoplasmic streaming (Huxley, 1973). Consider the large free-living amoebae Chaos carolinensis and Amoeba proteus. Cytoplasm isolated from these cells is highly contractile, resembling in its behaviour the supercontraction of

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muscle actomyosin threads (Taylor, Condeelis, Moore & Allen, 1973). Amoeba myosin thick filaments, with larger physical dimensions and more reactive enzymic sites than the monomer or corresponding globular Acanthamoeba (Pollard & Korn, 1973) myosin, would have a mechanical advantage in being capable of cross-linking more actin filaments and supporting higher tensions. This would result in the development of a greater degree of lateral organization of cytoplasmic actomyosin during contraction in a much shorter time. Thus, the existence of carnivorous amoeba myosin as thick filaments in vivo might be primarily responsible for organizing the massive bulk flow of cytoplasm characteristic of streaming in these organisms.

Factors influencing the state of aggregation of myosin and the distribution of the thick filaments in vivo might be responsible for controlling the site of motive force production, gradients of structure and the extent of the resultant movement. One such factor might be the local concentration of Mg$^{2+}$ and Ca$^{2+}$. Work with a variety of cytoplasmic myosins suggests that, in vivo, myosin thick filaments might be transient structures, the presence of which depends on the divalent cations present. Holberton & Preston (1970) demonstrated that glycerinated models of Amoeba discoides, contracted with Mg$^{2+}$ and ATP, contained large numbers of thick myosin-like filaments which were not observed prior to addition of the Mg$^{2+}$ and ATP. Similarly, Hinssen (1979) showed that thick filaments formed in actomyosin preparations from Physarum ageing in a buffer containing Mg$^{2+}$ and ATP. Nachmias (1972) demonstrated that partially purified Physarum myosin would form compact short filaments in the presence of millimolar Ca$^{2+}$, while highly purified Physarum myosin readily formed long filaments at low ionic strength in the presence of Mg$^{2+}$ and Ca$^{2+}$ (Nachmias, 1974). Stossel & Pollard (1973) demonstrated that myosin from polymorphonuclear leukocytes required Mg$^{2+}$ for thick-filament formation. The removal of divalent cations from the cytoplasm of Chaos carolinensis with EGTA disaggregates the myosin filaments (Condeelis, Taylor, Moore & Allen, 1976). It has been shown that the number of myosin filaments in cytoplasmic preparations of Amoeba proteus can be increased by adding a superthreshold Ca$^{2+}$ concentration (Taylor, Rhodes & Hammond, 1976a). If the aggregation and disaggregation of myosin thick filaments occurs in non-muscle systems in response to divalent cations it might constitute part of a novel control mechanism for cytoplasmic contractility.

However, it is not clear that the thick filament is necessary for the successful transduction of chemical energy into mechanical work. Assuming that most cytoplasmic myosin monomers contain 2 enzymic regions that are physically separated, as does muscle and Physarum myosin, cross-linking of adjacent but different F actin filaments by each amoeba subfragment could result in sliding of the actin filaments relative to one another. In addition, recent work by Oplatka and co-workers (Oplatka & Tiros, 1973; Oplatka, Gadasi & Borejdo, 1974a; Oplatka et al. 1974b) with chemically modified or enzymically produced subfragments of muscle myosin leads one to question the necessity of the thick filament or even a filamentous monomer in cytoplasmic streaming — an attractive possibility, considering the conceptual difficulties introduced by the discovery of the globular Acanthamoeba myosin (Pollard & Korn, 1973).

Because of these considerations, determination of the physical form of myosin in
Myosin filaments from Chaos and Amoeba has assumed new importance. Such information has been mainly derived from ultrastructural studies of fixed and sectioned cells. Recent work suggests that the presence or absence of myosin-thick filaments in fixed and sectioned cells is due as much to preservation artifact or section alignment (Komnick, Stockem & Wohlfarth-Bottermann, 1973; Niederman & Pollard, 1975) as to actual differences in the molecular properties of myosin from one organism to another. Consequently, the study of the formation of synthetic filaments from purified myosin at physiological ionic strength and pH has been a necessary tool in deriving information about the physical form of myosin in vivo.

This study was undertaken to determine the conditions necessary for self-assembly of myosin isolated from A. proteus and C. carolinensis under ionic strength and pH conditions believed to be physiological, and to determine the effect of the divalent cations Mg$^{2+}$ and Ca$^{2+}$ on aggregation.

MATERIALS AND METHODS

Protein preparation

*Amoeba proteus* and *Chaos carolinensis* were cultured, collected and their myosin extracted as previously described (Condeelis, 1977). Electrophoretically homogeneous myosin was collected from the agarose (Bio Rad A15m) chromatography column in 0.6 M KCl, 0.1 mM MgCl$_2$, 1 mM ATP, 1 mM dithiothreitol (DTT), 10 mM imidazole HCl, pH 7.0 and concentrated by either (NH$_4$)$_2$SO$_4$ precipitation or Millipore ultrafiltration at 4 °C.

Ammonium sulphate precipitation was carried out by dissolving the dry powder in the myosin solution to 50 % saturation and then centrifuging at 20000 g for 30 min. Millipore ultrafiltration was carried out using a 5.0 x 1.3 cm barrel apparatus (maximum volume = 3.0 ml) containing a 25000 nominal molecular weight limit filter at 2.76 x 10$^5$ KN m$^{-2}$ of nitrogen gas pressure. The ATPase activity and protein concentration were determined using micromethods described elsewhere (Condeelis, 1977). The concentrated myosin solution was adjusted to between 0.1 and 0.2 mg myosin/ml with high salt buffer (Table 1) and clarified in the ultracentrifuge at 100 000 g for 3 h.

Table 1. Solutions used for the study of self assembly of myosin thick filaments

<table>
<thead>
<tr>
<th></th>
<th>High salt buffer</th>
<th>EGTA-EDTA buffer</th>
<th>KCl buffer</th>
<th>Calcium buffer</th>
<th>Magnesium buffer</th>
<th>EGTA buffer</th>
<th>Ca$^{2+}$-EGTA buffer</th>
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<tbody>
<tr>
<td>KCl</td>
<td>300-0</td>
<td>300-0</td>
<td>50-0</td>
<td>50-0</td>
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<tr>
<td>CaCl$_2$</td>
<td>0-0</td>
<td>0-0</td>
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<td>0-0</td>
<td>1-0</td>
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<tr>
<td>MgCl$_2$</td>
<td>0-0</td>
<td>0-0</td>
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<td>EGTA</td>
<td>0-0</td>
<td>1-0</td>
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<td>0-0</td>
<td>0-0</td>
<td>2-0</td>
<td>1-0</td>
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<tr>
<td>EDTA</td>
<td>0-0</td>
<td>1-0</td>
<td>0-0</td>
<td>0-0</td>
<td>0-0</td>
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<td>0-0</td>
</tr>
<tr>
<td>Imidazole-HCl</td>
<td>10-0</td>
<td>10-0</td>
<td>10-0</td>
<td>10-0</td>
<td>10-0</td>
<td>10-0</td>
<td>10-0</td>
</tr>
<tr>
<td>pH</td>
<td>7-0</td>
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<td>7-0</td>
<td>7-0</td>
<td>7-0</td>
<td>7-0</td>
<td>7-0</td>
</tr>
</tbody>
</table>

Concentrations are given in millimolar

Formation of thick filaments

With each preparation of myosin 2 series of experiments were performed. The supernatant remaining after ultracentrifugation was divided in half and one half was dialysed against the
EGTA–EDTA buffer while the other was dialysed against high-salt buffer overnight (Table 1). These two samples were then further subdivided into 5 fractions of equal volume and each fraction was microdialysed (Condeelis, 1977) separately against one of the low ionic strength buffers in Table 1, overnight at 4 °C. These buffers had an ionic strength of approximately 0.05 M and a pH of 7.0. Since these conditions are believed to be physiological (Taylor et al. 1973) 0.05 M and pH 7.0 will be referred to as physiological ionic strength and pH. Experiments in which the myosin was predialysed against EGTA–EDTA buffer are referred to as predialysed myosin experiments, while those in which myosin was initially dialysed against high-salt buffer are referred to as column myosin experiments.

Muscle myosin was isolated as described by Kielly & Harrington (1960) and synthetic filaments were formed using the same method described above for amoeba myosin.

Electron microscopy

Protein solutions were applied to Formvar-coated grids and negatively stained according to the method of Moore, Condeelis, Taylor & Allen (1973), modified by rinsing residual buffer from the grid with water after fixation but before staining with uranyl acetate to reduce the background particulate size. All of the protein preparations were fixed by rinsing the grid with 2 % glutaraldehyde to avoid variation in filament dimensions due to shrinkage, flattening or increase in salt concentration during drying.

Grids were also prepared according to the method of Huxley (1963) to determine whether the rinsing involved in the Moore et al. (1973) technique would rinse the smaller thick filaments off the grid. The same results were obtained using both methods.

Measurements of the total length of the thick filament, length of the bare central zone and diameter at the centre of the bare central zone were made on the negatives. Observations were made with a Philips EM300 electron microscope with an accelerating voltage of 60 kV. A condenser aperture of 250 μm and an objective aperture of 50 μm were used. For all electron micrographs the microscope was calibrated at the time of use with a cross-ruled optical grating replica.

Determination of myosin solubility

After microdialysis against the various solutions shown in Table 1 the fractions were negatively stained and then centrifuged as previously described (Condeelis, 1977) at 80 000 g for 20 min in the same tubes as those in which dialysis was performed. The supernatants were assayed for ATPase and protein according to micromethods described elsewhere (Condeelis, 1977).

RESULTS

Amoeba myosin eluted from the agarose column at a concentration of ca. 0.04 mg/ml. In initial experiments thick filament formation occurred with myosin concentrated by either the ammonium sulphate or Millipore ultrafiltration methods. However, Millipore ultrafiltration resulted in a larger drop in the Ca²⁺ ATPase of the myosin, with a 40 % loss of the specific activity occurring when myosin was concentrated 10-fold starting at 0.02 mg/ml. It was found that the initial starting protein concentration, the degree of further concentration and the rate of filtration were all factors in preserving the specific activity of the myosin. The larger these factors, the lower the final specific activity. Ultrafiltration allowed over 90 % recovery of the protein initially present in the retentate.

Ammonium sulphate fractionation resulted in a larger loss of myosin to the supernatant, with as little as 50 % recovery of the total myosin available in the column effluent. However, less than 10 % of the specific activity was lost using the ammonium
Fig. 1. Thick filaments formed from column myosin dialysed against calcium buffer (Table 1). Filaments made from column myosin exhibited head-to-head and side-to-side interactions. Typically, 50 µl of solution containing 0.1 mg/ml myosin was divided into five 10-µl fractions and microdialysed overnight against the low ionic strength buffers in Table 1.
sulphate method. Therefore, ammonium sulphate precipitation was used to concentrate the myosin in these studies in order to avoid artifacts of filament formation that might result with inactive myosin monomer.

Table 2. Influence of buffer conditions on the formation of filaments from column myosin

<table>
<thead>
<tr>
<th>Buffer*</th>
<th>Total length, nm</th>
<th>Bare central zone, nm</th>
<th>Diameter, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>433 ± 49</td>
<td>175 ± 37</td>
<td>140 ± 50</td>
</tr>
<tr>
<td></td>
<td>(365-520)</td>
<td>(130-220)</td>
<td>(7±20)</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>463 ± 35</td>
<td>184 ± 17</td>
<td>167 ± 53</td>
</tr>
<tr>
<td></td>
<td>(415-540)</td>
<td>(156-210)</td>
<td>(104±23)</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>461 ± 42</td>
<td>155 ± 13</td>
<td>167 ± 41</td>
</tr>
<tr>
<td></td>
<td>(415-520)</td>
<td>(130-182)</td>
<td>(108±20)</td>
</tr>
<tr>
<td>EGTA</td>
<td>475 ± 35</td>
<td>192 ± 16</td>
<td>197 ± 21</td>
</tr>
<tr>
<td></td>
<td>(415-520)</td>
<td>(167-218)</td>
<td>(156±22)</td>
</tr>
<tr>
<td>EGTA–Ca²⁺</td>
<td>444 ± 25</td>
<td>164 ± 17</td>
<td>152 ± 3</td>
</tr>
<tr>
<td></td>
<td>(415-470)</td>
<td>(143-168)</td>
<td>(148±20)</td>
</tr>
<tr>
<td>High salt buffer†</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

The number of filaments measured per experiment was 30.

Column myosin was dialysed against high salt buffer (Table 1) before being divided into fractions for dialysis against the low ionic strength buffers. Predialysed myosin was dialysed against EGTA-EDTA buffer (Table 1) before final dialysis against the low ionic strength buffers.

* These solutions refer to those listed in Table 1.
† No filaments were observed.

Filament-formation with column myosin

Typical results obtained when column myosin was dialysed against the calcium buffer (Table 1) are shown in Fig. 1. Indistinguishable results were obtained with myosin isolated from C. carolinensis and A. proteus and the dimensions of the filaments from either species of myosin are averaged in Tables 2 and 3. Dialysing column myosin against any of the low ionic strength buffers in Table 1 resulted in filaments with almost identical morphology (Fig. 2) and dimensions (Table 2). In all of these preparations the grids were heavily littered with thick filaments exhibiting end-to-end and side-to-side interactions, with fewer free non-interacting thick filaments scattered about. The synthetic filaments closely resembled in dimensions and morphology the native myosin filaments observed in negatively stained cytoplasm isolated from amoebae (Moore et al. 1973; Condeelis et al. 1976; D’Haese & Hinssen, 1974). However, in these solutions synthetic filaments formed from column myosin had their heads predominantly clumped together on the surface of the backbone of the filament (Figs. 1, 2), in contrast to thick filaments in crude cytoplasmic preparations, in which heads were observed spread out away from the filaments with equal frequency (Condeelis et al. 1976). The average dimensions of the synthetic filaments were 455 nm long, 16 nm wide with a distinct bare central zone 174 nm long.

When monomer was dialysed against the high salt buffer, filament formation did not
Fig. 2. An example of the morphology of the thick filaments formed by dialysing column myosin against 50 mM KCl, 10 mM imidazole-HCl, pH 7-0 containing:
A, KCl only; B, CaCl₂; C, MgCl₂; D, EGTA; E, EGTA-Ca²⁺ (Table 1). Scale = 0.5 μm.

Fig. 3. Column myosin monomer dialysed against high salt buffer (300 mM KCl, 10 mM imidazole, pH 7-0) failed to form thick filaments.
Filament formation with predialysed myosin

Various investigators have reported a divalent cation requirement for thick filament formation with myosin from several organisms (Nachmias, 1972, 1974; Stossel & Pollard, 1973). Therefore, experiments were designed to test for such a requirement in carnivorous amoeba myosin.

During purification carnivorous amoeba myosin was eluted from the column in the presence of Mg\(^{2+}\). It was possible that residual Mg\(^{2+}\) ions, bound to the myosin monomer, would result in thick filament formation in the absence of exogenous Mg\(^{2+}\). By dialysing against EGTA–EDTA buffer overnight it was hoped the residual Mg\(^{2+}\) ions would be removed and a better estimate of the divalent ion requirement for filament formation could be made.

Under these conditions thick filament formation occurred in all of the low ionic strength buffers in Table 1, but only the filaments grown in the presence of MgCl\(_2\) (Fig. 4) resembled typical synthetic filaments observed in the column myosin experiments (Figs. 1, 2) and native myosin filaments observed in amoeba cytoplasm (Moore et al. 1973; Condeelis et al. 1976; D'Haese & Hinssen, 1974).

Filaments grown in all other buffers in the absence of MgCl\(_2\) resulted in poorly formed, highly branched filaments lacking a distinct bare central zone as shown in Fig. 5. Unexpectedly, even the two concentrations of Ca\(^{2+}\) tested could not be substituted for MgCl\(_2\) in the production of compact native-like filaments (Fig. 6). The average dimensions of the filaments grown in the absence of MgCl\(_2\) were 328 nm long and 13 nm wide, with a bare central zone 111 nm long. These filaments were smaller than those grown in the presence of MgCl\(_2\) which had dimensions similar to the filaments made from column myosin. These results are summarized in Fig. 7 and Table 3.

Samples dialysed against low ionic strength buffers in the absence of MgCl\(_2\) contained numerous smaller filaments that were not considered when averaging the dimensions of the filaments in the preparations (Fig. 5, arrow). These thin filaments did not bind amoeba myosin monomer and might, therefore, be smaller oligomers of myosin.

Filaments grown from predialysed myosin in the absence of Mg\(^{2+}\) exhibited a greater tendency to clump into aggregated masses (Figs. 5, 6). Because of the spread morphology and clumping of the filaments in these preparations, the measurements in Table 3 must be viewed with caution, as these effects produced difficulties in making accurate measurements in some cases. Filaments grown in the presence of MgCl\(_2\) or with column myosin were always more separated, with interactions confined to end-to-end and side-to-side arrangements (Figs. 1, 4).

Muscle myosin control experiments

In order to compare the filament-forming properties of myosin monomer from amoebae and rabbit skeletal muscle directly, muscle myosin, at a concentration of 0.1
Fig. 4. Thick filaments formed in the presence of magnesium buffer from pre-dialysed myosin (Table 1). Filaments grown under these conditions resembled native thick filaments and filaments formed from column myosin.

Fig. 5. Thick filaments formed in the presence of EGTA buffer (Table 1) from pre-dialysed myosin. A few short, thin, non-actin filaments (arrow) were also present in these preparations and are probably smaller oligomers of myosin.
Fig. 6. Thick filaments formed in the presence of calcium buffer from predialysed myosin (Table 1). Ca\(^{2+}\) could not be substituted for Mg\(^{2+}\) in producing tightly packed thick filaments from predialysed myosin.

Fig. 7. Survey of the thick filaments formed from predialysed myosin in the presence of 50 mM KCl, 10 mM imidazole - HCl, pH 7.0 containing: A, KCl only; B, CaCl\(_2\); C, MgCl\(_2\); D, EGTA; E, EGTA-Ca\(^{2+}\); F, EGTA (rabbit skeletal muscle myosin). Scale = 0.5 \(\mu m\).
Table 3. Influence of buffer conditions on the formation of filaments from predialysed myosin

<table>
<thead>
<tr>
<th>Buffer*</th>
<th>Total length, nm</th>
<th>Bare central zone, nm</th>
<th>Diameter, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>353 ± 30</td>
<td>106 ± 17</td>
<td>14.9 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>(312-390)</td>
<td>(78-130)</td>
<td>(9.8-20.8)</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>318 ± 76</td>
<td>103 ± 17</td>
<td>12.9 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>(210-400)</td>
<td>(84-138)</td>
<td>(8.0-20.8)</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>425 ± 61</td>
<td>163 ± 18</td>
<td>16.3 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>(344-520)</td>
<td>(140-208)</td>
<td>(10.8-20.8)</td>
</tr>
<tr>
<td>EGTA</td>
<td>289-52</td>
<td>84 ± 18</td>
<td>12.7 ± 3.0</td>
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<tr>
<td></td>
<td>(210-365)</td>
<td>(62-120)</td>
<td>(7.8-17.0)</td>
</tr>
<tr>
<td>EGTA–Ca²⁺</td>
<td>352 ± 69</td>
<td>152 ± 25</td>
<td>12.7 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>(210-430)</td>
<td>(78-208)</td>
<td>(8.2-20.8)</td>
</tr>
</tbody>
</table>

The number of filaments measured per experiment was 30.

Column myosin was dialysed against high salt buffer (Table 1) before being divided into fractions for dialysis against the low ionic strength buffers. Predialysed myosin was dialysed against EGTA–EDTA buffer (Table 1) before final dialysis against the low ionic strength buffers.

* These solutions refer to those listed in Table 1.

mg/ml, was predialysed overnight against EGTA–EDTA buffer followed by dialysis against the same low ionic strength buffers used in Table 1. Under these conditions muscle myosin formed tapered filaments over 1 μm in length the morphology of which was typical of that described by Huxley (1963) for synthetic myosin filaments (Fig. 8). A direct size comparison between amoeba and muscle myosin-thick filaments is shown in Fig. 7.

Myosin solubility

Myosin was easily sedimented by centrifugation at 80,000 g for 30 min after dialysis against the low ionic strength buffers in Table 1. The percentage of myosin sedimented was estimated to be ca. 75 % by protein assay and ca. 85 % by ATPase assay. Similar results were obtained with all of the low ionic strength buffers tested in Table 1 using both column and predialysed myosin, which suggests that thick filament formation occurred to an approximately equal degree in each preparation.

DISCUSSION

The filament-forming properties of myosin from A. proteus and C. carolinensis can be summarized as follows. (1) At physiological ionic strength and pH, amoeba myosin filament assembly does not exhibit an absolute requirement for divalent cations. This property is shared by Dictyostelium myosin (Clarke & Spudich, 1974), while myosin isolated from Physarum (Nachmias, 1974) and polymorphonuclear leukocytes (Stossel & Pollard, 1973) requires divalent cations for pronounced thick filament formation. (2) Buffers containing up to 1 mM CaCl₂ are no more effective in producing native-like thick filaments than buffers containing EGTA. Thus, Ca²⁺ cannot be substituted...
Fig. 8. Thick filaments formed in the presence of EGTA buffer (Table 1) from predialysed rabbit skeletal muscle myosin.
for Mg^{2+} in producing compact native-like filaments made from predialysed myosin. (3) Thick filaments formed from predialysed myosin in the absence of Mg^{2+} are highly branched, poorly formed filaments that resemble the EGTA-dissociated native filaments observed in amoeba cytoplasm (Condeelis et al. 1976). Such preparations contain numerous oligomers of lower order that appear to take part in the cross-linking of aggregates formed in the absence of Mg^{2+}. Mg^{2+} apparently alters the interactions between monomers, leading to aggregates with fewer interaggregate interactions and a reduction of clumping of the thick filaments. (4) Carnivorous amoeba myosin formed thick filaments that were smaller than and different in morphology from the thick filaments made from rabbit skeletal muscle myosin under precisely the same conditions. Muscle myosin filament formation was unaltered in the presence or absence of Ca^{2+}, Mg^{2+} or EGTA. These results indicate basic differences in the molecular properties of these two species of myosin. (5) The ability of carnivorous amoeba myosin to form thick filaments and/or the morphology of the filaments formed is dependent on the history of the myosin monomer. This is an important consideration when dealing with myosin of unknown properties and it may account for some of the differences observed in the solubility properties of myosin from different sources. The treatment of the monomer before the thick filaments are grown (e.g. dialysis against EGTA or EDTA) may be as decisive in determining the morphology or divalent cation requirements of the final product as ionic strength and pH.

With this information it is now possible to speculate on the physical form assumed by amoeba myosin in vivo. All conclusions must consider the following observations. (1) Ultrastructural investigations on fixed, thin-sectioned intact amoebae have shown the presence of thick myosin-like filaments (Nachmias, 1968; Comly, 1973). (2) Fixed, thin-sectioned amoeba models capable of vigorous contractility at the time of fixation contain thick filaments (Taylor et al. 1973; Moore, 1975). (3) Cytoplasm freshly isolated from amoebae contains numerous native thick filaments (Moore et al. 1973; Condeelis et al. 1976; D’Haese & Hinssen, 1974) of approximately the same dimensions as those observed in thin-sectioned material and in preparations of synthetic filaments formed in the presence of Mg^{2+}. (4) Millimolar amounts of magnesium have been shown to exist in the giant amoebae (Friz, 1971). Since free Mg^{2+} is a necessary ionic component of solutions producing contraction and relaxation of amoeba cytoplasm (Taylor et al. 1973), it is likely that much of this magnesium is present in vivo in dissociated form. (5) Further studies on the ionic requirements for contraction of amoeba cytoplasm led to the observation that, in the presence of millimolar concentrations of EGTA, the optical retardation, gross organization and contractile response of cytoplasmic fibrils decreased (Taylor, Moore, Condeelis & Allen, 1976b). An ultrastructural investigation of this phenomenon led to the observation that native amoeba myosin and actin filaments are labile in the absence of divalent cations (Condeelis et al. 1976). The actin filaments were especially unstable at physiological ionic strength, completely depolymerizing in the absence of myosin and divalent cations within several minutes. The myosin thick filaments were much more stable, requiring high concentrations of EGTA and longer incubation times for disaggregation.
It appears attractive to suggest that in regions of the amoeba in which the Ca$^{2+}$ concentration is reduced the actin and myosin filaments might depolymerize, resulting in loss of cytoplasmic structure and contractility. This would be important in developing gradients of viscoelasticity and contractility postulated to be necessary for movement in the giant amoebae (Allen, 1972; Taylor et al. 1973). However, in view of the present findings one might conclude that the myosin thick filament is stable in the presence of in vivo levels of Mg$^{2+}$ and it would not be affected by changes in the Ca$^{2+}$ concentration, at least in the range postulated to occur in vivo ($10^{-9} - 10^{-6}$ M) (Taylor et al. 1973). This is consistent with observations on the stability of native myosin thick filaments in cytoplasmic preparations in the presence of EGTA (Condeelis et al. 1976).

If filament polymerization-depolymerization is important for streaming in amoeba cytoplasm it might be the actin that is involved. Carnivorous amoeba F actin is extremely unstable, depolymerizing readily at low divalent cation concentrations (Condeelis et al. 1976). Thus it might play an important role in the control of structure and contractility of amoeba cytoplasm by polymerizing in the presence of suprathreshold Ca$^{2+}$ concentrations and depolymerizing in the presence of subthreshold Ca$^{2+}$ concentrations.

A note of caution is necessary here regarding conclusions about the physical state of myosin in vivo. Ultrastructural studies on fixed thin-sectioned cells or biochemical studies of the self-assembly of purified myosin cannot, in themselves, resolve the question of the filamentous state of myosin in vivo. Thin sectioning is given to artifacts of alignment (Niederman & Pollard, 1975) and gives no information on the equilibrium of myosin between its monomeric and aggregated states before fixation. Biochemical studies are compromised because, in the cell, the presence of auxiliary proteins may substantially alter the aggregation behaviour of myosin and the stability of the myosin thick filament. Since these proteins might be separated from the myosin during purification, different self-assembly behaviour might be expected, depending on the relative purity of the myosin. Nachmias (1972, 1974) has observed that Physarum myosin filament formation is dramatically affected by the degree of purification of the myosin. Furthermore, D’Haese & Hinssen (1974) have observed synthetic filament formation in crude preparations of myosin prepared from A. proteus containing many uncharacterized components. The thick filaments grown under these conditions were much larger than those reported here and demonstrated a structural asymmetry in the bare central zone that was not apparent in the thick filaments grown from purified A. proteus myosin reported here.

These differences in the filament-forming properties of myosin from Physarum and A. proteus may be due to either differences in the preparation and handling of the myosin, which might have an effect on the intrinsic properties of the molecule, or the presence of contaminating proteins which might bind to myosin and affect its solubility properties. In either case, as observed above, the history of the myosin monomer must be considered when drawing conclusions about the relative solubility properties of myosin from different organisms or even from the same organism prepared by different methods. This suggests that it would be valuable to study the solubility properties of
Myosin filaments from Chaos and Amoeba

myosin and thick filament morphology in vitro at various stages of purification in order to estimate these properties realistically in vivo.

Finally, the data available at present indicate that the presence of auxiliary proteins may substantially affect the properties of actin and myosin. Indeed, the control of cytoplasmic structure and contractility might result from the interaction of auxiliary proteins with actin and myosin resulting in the formation of structures that are capable of contraction and movement (Condeelis & Taylor, 1977; Kane, 1975; Pollard, 1976; Stossel & Hartwig, 1976; Taylor et al. 1976a; Tilney, 1975).

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