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Growth of Synthetic Myosin Filaments from Myosin Minifilaments†

Emil Reisler,* Pearl Cheung, Christine Oriol-Audit,‡ and James A. Lake

ABSTRACT: Addition of KCl to a solution of synthetic myosin minifilaments in 10 mM citrate–Tris buffer (pH 8.0) induces the growth of filaments. These filaments, at pH 8.0, resemble in their morphological and hydrodynamic properties the synthetic filaments described by Josephs and Harrington [Josephs, R., & Harrington, W. F. (1966) *Biochemistry 5*, 3474–3487]. The rate of filament growth depends critically on the KCl concentration in the solution. Low rates of filament formation are noted in the presence of both low (below 80 mM KCl) and high (above 0.15 M KCl) salt concentrations, whereas at the intermediate KCl concentrations the filaments are formed at a fast rate. The formation of filaments from minifilaments is a reversible process, and under moderate salt concentrations, these two polymeric systems appear to exist in a dynamic equilibrium. Small amounts of minifilaments can induce rapid polymerization of dissociated myosin; i.e., they can act as a seeding material. These and other observations are discussed in terms of a direct route for filament formation from myosin minifilaments.

The electron microscopy studies of Huxley (1963) established the structural similarities of native myosin filaments and the synthetic filaments obtained by decreasing the ionic strength of a myosin solution. These findings implied that the same principles might govern the assembly of both filaments. The bipolar character of myosin filaments, and the presence of a smooth central region in these structures, indicated a two-step process of their formation: initial antiparallel (tail to tail) aggregation of myosin molecules to form short bipolar structures, and the subsequent addition of myosin in a parallel fashion (head to tail) at each end of the bipolar unit (Huxley, 1963; Kaminer & Bell, 1966; Josephs & Harrington, 1966; Huxley & Brown, 1967; Pepe, 1967; Harrington, 1972; Katsura & Noda, 1971, 1973; Hinssen et al., 1978). Although this model of assembly is widely accepted, no detailed study has been undertaken to confirm it. Until now, very little is known about the formation of myosin filaments and the factors which determine their length.

Most of the previous work on myosin filaments was concerned with their structural properties and the effect of experimental parameters on the final assembly products. The composition and myosin content of the thick filaments have

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been intensely investigated (Morimoto & Harrington, 1973, 1974; Rome et al., 1973; Pepe & Drucker, 1979; Craig & Offer, 1976; Lamvik, 1978; Emes & Rowe, 1978) in order to obtain the necessary information for evaluating the packing of myosin in the thick filament (Squire, 1973, 1975; Pepe, 1967, 1979; Huxley & Brown, 1967). The structural information on myosin packing may eventually come from studies on the mechanism of filament assembly. Previous work in this area has provided important insight into the effects of pH, ionic strength, protein concentration, the rate of reducing the ionic strength, and the presence of C-protein on the size distribution of synthetic myosin filaments (Huxley, 1963; Kaminer & Bell, 1966; Josephs & Harrington, 1966, 1968; Katsura & Noda, 1971, 1973; Pepe, 1979; Moos, 1972; Koretz, 1979a,b). In most cases the synthetic filaments displayed large size heterogeneity, in contrast to the native filaments, which distribute narrowly around the average length of 1.6 μm. The most homogeneous synthetic preparations had an average particle length of 0.63 μm and were obtained by dialyzing myosin (in 0.5 M KCl) into the 0.12 M KCl, pH 8.0 solvent. More recently, Pepe (1979) succeeded in preparing homogeneous synthetic filaments (at pH 7.0) with lengths in the range of 1.5 $\pm$ 0.2 μm.

In the studies reported up to now, with the exception of Katsura & Noda (1971, 1973), the process of filament formation has not been monitored. The fast rate of myosin aggregation upon lowering the ionic strength and the apparent complexity of the assembly reaction discouraged detailed investigations of filament formation. The results of turbidometric stop-flow experiments of Katsura & Noda (1971, 1973) were consistent with a nucleation-growth scheme for filament assembly on the assumption that the myosin dimer was the basic building unit in this reaction.

The recently described myosin minifilaments (Reisler et al., 1980) appear to be particularly relevant and important to filament assembly studies. The stabilization (at pH 8.0) of minifilaments, which are composed of 16–18 myosin molecules and have a bipolar appearance corresponding to the central region of the thick filament, provides experimental evidence for the existence of different bonding interactions along the filament structure. It shows that the minifilaments are more stable than the larger myosin aggregates and suggests that their formation may consitute the first step in filament assembly.

The results presented in this report document the formation of synthetic myosin filaments due to the addition of KCl to solutions of minifilaments (at pH 8.0). Using electron microscopy and ultracentrifugation, we show that filaments formed in such manner have the molecular properties characteristic of myosin filaments (at pH 8.0) described by Josephs & Harrington (1966). Next, we explore in some detail the process of filament growth from the myosin minifilaments and demonstrate that this reaction can be easily and well regulated by fine adjustments of the ionic strength conditions in the medium. Consequently, this reaction can be conveniently monitored by various experimental techniques. As a result of these experiments, we conclude that myosin minifilaments constitute a convenient and promising system for studies on myosin filament formation.

Materials and Methods

Distilled water and analytical-grade reagents were used in all experiments. Dimethylsuberimidate was purchased from Pierce Chemical Co. (Rockford, IL).

Preparation of Myosin. Rabbit myosin was prepared and stored as described previously (Godfrey & Harrington, 1970). Before use, myosin was exhaustively dialyzed against 0.6 M KCl, 10 mM sodium phosphate (pH 7.0), and 0.1 mM NaN_3 and clarified by centrifugation at 100000*g* for 2 h. No attempt was made to remove the C-protein.

Preparation of Synthetic Minifilaments. Solutions of minifilaments were prepared as previously described (Reisler et al., 1980). In brief, myosin in 0.6 M KCl and 10 mM phosphate (pH 7.0) was dialyzed against 5 mM sodium pyrophosphate (pH 8.0), then clarified by centrifugation at 27000*g* for 30 min, and redialyzed against 10 mM citrate–Tris buffer (8.0). The final minifilament preparation in citrate–Tris buffer was reclarified by centrifugation (at 27000*g* for 20 min).

Concentration of minifilament solutions was determined spectrophotometrically after dissociation of the polymeric system into monomeric myosin (by dilution to 0.5 M KCl).

Ultracentrifugation Experiments. Sedimentation velocity experiments were carried out at rotor temperatures close to 20 °C in a Spinco Model E analytical ultracentrifuge. At high concentrations of protein (above 1.5 mg/mL), the sedimentation process was monitored by using the schlieren optical system, whereas at low protein concentrations the sedimentation was measured with the photoelectric scanning system of the Model E ultracentrifuge. Most of the sedimentation velocity experiments were performed at rotor speeds of (15–30) $\times 10^3$ rpm. The calculated sedimentation coefficients were reduced to standard conditions of water at 20 °C.

Determinations of myosin "monomer–dimer" concentration in the presence of filaments (in 0.1 M KCl) were made by centrifugation. After the filament and monomer boundaries were resolved, the absorbance across the "monomer region" of the cell (at 280 or 230 nm) was used as a concentration measure. The filament concentration was obtained from the total protein and monomer concentrations.

Turbidity Measurements. Turbidity measurements were made in a Beckman Model 25 spectrophotometer by monitoring optical density readings at 310 nm. The turbidities of myosin minifilaments and filaments obeyed Beer's law up to a protein concentration of 0.3%. Stock solutions of KCl and other ligands were made in citrate–Tris buffer and added in small volumes to protein solutions.

Electron Microscopy. Myosin minifilaments and filaments were routinely cross-linked with dimethylsuberimidate prior to electron microscope studies. The cross-linking reaction was carried out for 2 h, at 0 °C, and at a final protein concentration of 0.5 or 1 mg/mL. The final reagent concentration (dissolved in citrate–Tris buffer and adjusted to pH 8.0) was 1 mg/mL. The cross-linking was terminated by the addition to the reaction system of 0.2 volume of 2 M ethanolamine. Reactions carried out under these conditions were essentially limited to intrafilament cross-linking. This was suggested by the fact that the turbidity of the polymeric system changed very little during the cross-linking reaction.

Electron micrographs of cross-linked protein samples were taken at an operating voltage of 80 kV on a Philips 400 microscope. Samples were negatively contrasted by a double-layer carbon procedure described by Lake (1979). Length measurements of the myosin minifilaments and filaments were made directly from the electron micrographs, at a final magnification of 215000 diameters, by using an electronic measuring device (Griffith & Kornberg, 1974).

Results

KCl Induces an Association Reaction in Solutions of Myosin Minifilaments. In previous work (Reisler et al., 1980) we have discussed the molecular properties, the homogeneity, and the stability of the synthetic myosin minifilaments. We

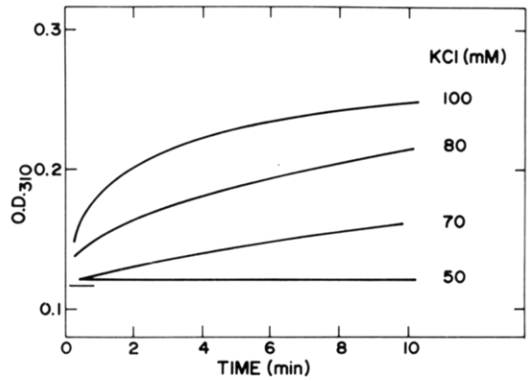

FIGURE 1: Changes in the turbidity of synthetic myosin minifilaments with time, following the addition of KCl to final concentrations as specified in the figure. The turbidity of the KCl-free solution of minifilaments is indicated by a bar next to the OD_{310} axis. Protein concentration is 2 mg/mL; solvent is 10 mM citrate–Tris, pH 8.0.

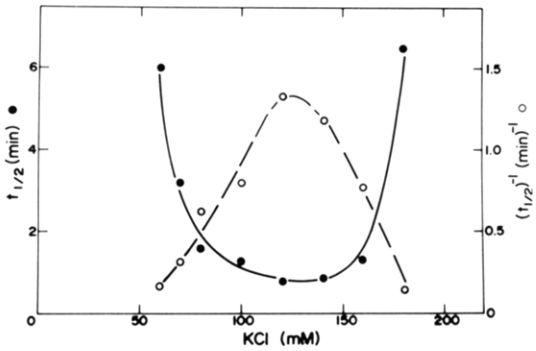

FIGURE 2: Time ($t_{1/2}$) required to achieve 50% of the turbidity change that occurs within 30 min after addition of various amounts of KCl to solutions of myosin minifilaments (2 mg/mL). The reciprocal value of this half-time, $t_{1/2}^{-1}$, is indicative of the net rate of filament formation. Solvent is the same as in Figure 1.

have noted that, in the absence of KCl, solutions of minifilaments do not change signficantly over a period of several days. Long-term stability of myosin minifilaments has not been demonstrated in the presence of low salt concentrations, between 0 and 40 mM KCl, but we have not detected notable changes in their properties within 2 h after the addition of KCl (Reisler et al., 1980; Reisler, 1980). This latter stability feature of myosin minifilaments is particularly important for their utilization in catalytic studies of myosin and actomyosin ATPase reactions.

The apparent stability of myosin minifilaments does not extend into solutions of higher salt concentrations. In the presence of 0.1 M KCl, the turbidity of minifilament solutions increases rather rapidly (Reisler et al., 1980). A detailed account of salt-induced changes in the turbidity of myosin minifilaments is presented in Figure 1. It can be seen that the initial rates of turbidity increase (at constant protein concentration) are profoundly affected by the final salt concentration in the minifilament solution. These rates increase with an increase in KCl levels up to 0.13 or 0.14 M KCl and decrease at higher salt concentrations. This striking effect of salt on the rate of turbidity increase is presented in Figure 2 in the form of the time ($t_{1/2}$) required for completion of 50% of the turbidity change at a given KCl concentration. In this context $t_{1/2}$ is a purely operational and comparative value since the changes in turbidity follow neither first- nor second-order kinetics. Both at low (40 mM) and at high (0.2 M) KCl concentrations no filament formation is detected. In low salt this appears to be due to the stability of minifilaments, whereas in high salt no net production of filaments is seen because of

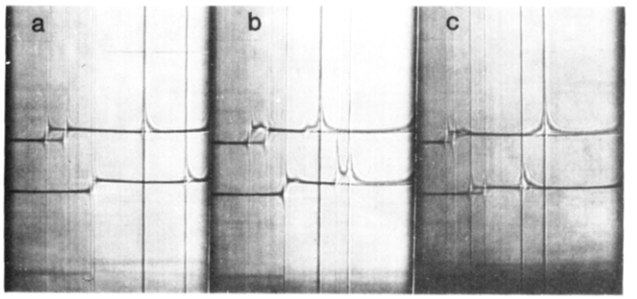

FIGURE 3: Velocity sedimentation patterns of synthetic myosin minifilaments in the presence of KCl. Solvent is the same as in Figure 1; temperature, 20–21 °C; protein concentration, 3 mg/mL; rotor speeds, 20 000 and 24 000 rpm. (a) Myosin minifilaments in the absence of KCl, $s_{20,w}$ = 17.5 S (lower, regular cell); myosin minifilaments in the presence of 20 mM KCl, $s_{20,w}$ = 17.6 S (upper, wedge cell). (b) Protein as in (a) plus 80 (lower, regular cell) and 100 mM KCl (upper, wedge cell). The respective sedimentation coefficients, 2 h after the addition of salt, are in the order of the increasing migration rate 6.2 and 46.0 S for the wedge cell and 6.1, 28.2, and 41 S for the regular cell. (c) Protein as in (a), after 24-h dialysis against 10 mM citrate–Tris, pH 8.0, containing either no KCl (lower, regular cell) or 0.1 M KCl (upper, wedge cell). The respective $s_{20,w}$ values are 6.1 and 66 S for the wedge cell and 17.2 S for the regular cell.

their instability under such solvent conditions. Filament instability is easily demonstrated by adding KCl, to a final concentration of 0.2 M, into a solution of filaments grown at a lower salt level. This leads to filament dissociation and the loss of turbidity. It should be noted that the stabilities of the polymeric structures are probably determined by kinetic effects, i.e., the rates of the competing dissociation and association reactions.

At a given salt concentration, the initial rates of turbidity increase (OD_{310}/min) depend linearly on the total protein concentration. Protein concentration, and to some extent KCl levels, determines, also, the final equilibrium OD_{310} values attained in such solutions. A possible explanation of this latter salt effect will become apparent later in the text.

In principle, turbidity measurements can be related to the concentration and the size of polymeric species present in the solution. However, the simplest version of such measurements, that of spectrophotometric OD readings at a single wavelength, does not yield any relevant size information on solutions containing large structures. Thus, the time-dependent turbidity profiles shown in Figure 1 (and those used in constructing Figure 2) should be merely viewed as qualitative evidence for the formation of larger myosin aggregates upon the addition of salt to solutions of minifilaments.

Synthetic Filaments Are Grown in Solutions of Minifilaments. A better insight into the myosin association process is provided by analytical ultracentrifugation studies. Figure 3 shows some typical sedimentation velocity profiles for solutions of minifilaments in the presence and absence of KCl. As previously reported (Reisler et al., 1980), myosin minifilaments prepared in citrate–Tris buffer (pH 8.0), and in the absence of KCl, sediment in the form of a hypersharp bar (Figure 3a). The same sedimentation profiles and coefficients (17.5 S) are observed 2 h after addition of 20 or 30 mM KCl to minifilaments (Figure 3a). In both cases, with and without salt, no other myosin species except for minifilaments can be detected in the solution.

A very different sedimentation profile is observed 2 h after the salt concentration in the minifilament solution is brought up to 80 or 100 mM KCl (Figure 3b). In the presence of 80 mM KCl, three peaks can be easily seen. The slowest peak ($s_{20,w}$ = 6.1 S) migrates at the rate of monomeric myosin, the

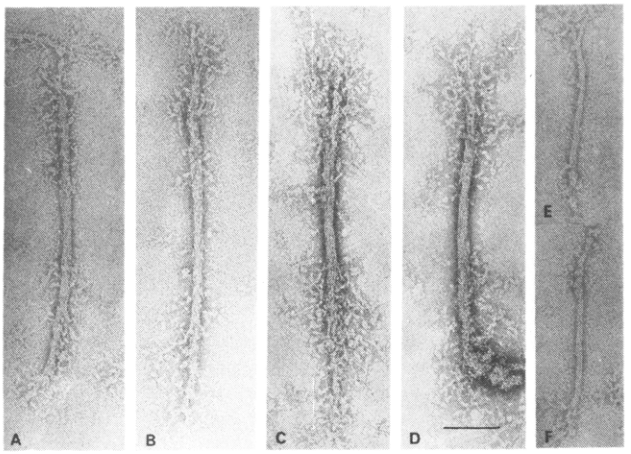

FIGURE 4: Cross-linked synthetic myosin filaments (A–D) and minifilaments (E, F). The filaments were obtained by dialyzing a solution of minifilaments in 10 mM citrate–Tris buffer (pH 8.0) against 0.1 M KCl and 10 mM citrate–Tris solvent. All pictures are taken at the same magnification. The bar length corresponds to 0.1 μm.

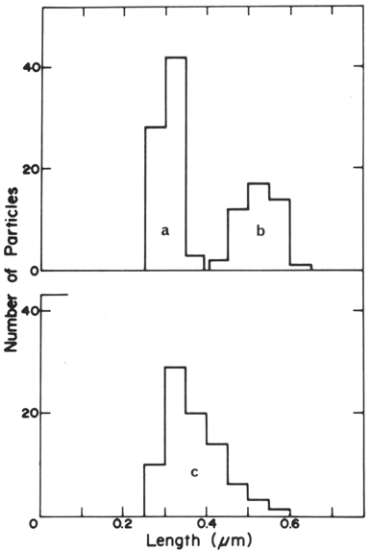

FIGURE 5: Histogram of distribution of lengths of myosin particles. (a) Myosin minifilaments in 10 mM citrate–Tris buffer (pH 8.0). (b) Myosin filaments formed by dialyzing minifilaments against 0.1 M KCl and 10 mM citrate–Tris pH, 8.0. (c) Minifilament–filament system 48 h after addition of 70 mM KCl to a solution of minifilaments. In all cases myosin samples were cross-linked at a protein concentration of 1 mg/mL prior to their dilution for electron microscope observations.

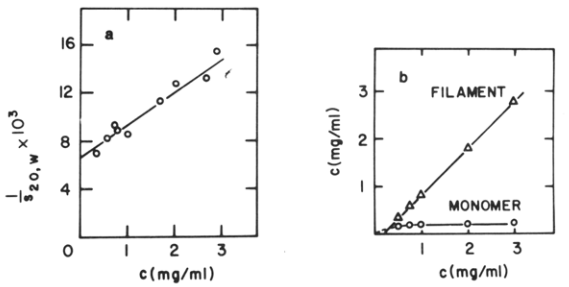

FIGURE 6: Sedimentation properties of myosin filaments grown by dialyzing the minifilaments against 0.1 M KCl and 10 mM citrate–Tris buffer (pH 8.0). (a) Sedimentation velocity of filaments vs. total myosin concentration. (b) Concentration of filaments and monomer–dimer species as a function of the total myosin concentration (these concentrations were determined as described under Materials and Methods).

next one ($s_{20,w}$ = 28 S) appears to correspond to the minifilament species, and the fastest peak ($s_{20,w}$ = 40 S) reveals the presence in solution of structures larger than the minifilaments. As documented in subsequent figures, these are the myosin filaments. It should be noted that the " minifilament" and "filament" peaks shown in Figure 3b cannot be fully resolved under any conditions. In fact, the boundary profile in this run is characteristic of a reaction boundary and indicates heterogeneity of polymer sizes in the centrifuged sample. At the higher salt concentration (100 mM KCl), consistent with the observed faster rate of turbidity change (Figure 1), most of the myosin minifilaments have been converted into the filament species ($s_{20,w}$ = 46 S). When reexamined after an overnight dialysis against 0.1 M KCl, this system contains only monomer and filament species and virtually no minifilaments (Figure 3c). In the lower salt solution (80 mM KCl), the minifilament to filament transition is not completed after a similar time, although a significant redistribution of myosin occurs from the slower to the faster peak (not shown). Within the same time interval, the sedimentation rate of a control minifilament does not change (Figure 3c).

The formation of filaments from minifilaments is most convincingly demonstrated by electron microscopy studies. The micrographs and histograms presented in the next figures may be assumed to be snapshots of the myosin assembly system, at 1 mg/mL, in the presence of 70 and 100 mM KCl. The original size distributions of myosin polymers were "frozen" by cross-linking the protein with suberimidate. Cross-linkings at higher protein concentrations were attempted and abandoned because of detectable interfilament cross-linking. Figure 4 presents a gallery of representative high-magnification pictures of filaments obtained after the addition of 0.1 M KCl to myosin minifilaments. For comparison, two minifilaments are also included in this gallery. The most important conclusion that can be derived from visual inspection of these and many other micrographs is that, indeed, addition of KCl to myosin minifilaments results in the formation of synthetic filaments. (Neither turbidity nor sedimentation experiments alone could exclude the possibility of nonspecific aggregation of minifilaments.)

The size distributions shown in Figure 5 shed additional light on the assembly process under consideration. As previously reported (Reisler et al., 1980), the original minifilament preparations (in the absence of KCl) are highly homogeneous and contain particles with an average length of 0.31 μm (±0.02 μm). On the other side of the size spectrum are the filaments formed by addition of 0.1 M KCl to the minifilaments. Solutions of such filaments appear homogeneous by criteria of sedimentation velocity profiles and display a rather narrow size distribution in the histogram shown in Figure 5. The average length of the filament particles is 0.52 μm (±0.048 μm). No minifilaments and very few, if any, particles of intermediate size (between the minifilaments and the filaments) can be detected in the electron micrographs of filaments grown in 0.1 M KCl. In terms of their size and properties, these filaments closely resemble the synthetic filaments prepared by Josephs & Harrington (1966) by directly dialyzing monomeric myosin (in 0.5 M KCl) into 0.12 M KCl, pH 8.0 solvent. In the previous work (Josephs & Harrington, 1966) and this paper, the synthetic filaments have an intrinsic sedimentation coefficient $s_{20,w}$ = 150 S and appear to be in a rapid equilibrium with monomer–dimer species (Figure 6). Also, as shown before (Josephs & Harrington, 1966), above certain threshold protein concentrations, the monomer–dimer concentration remains constant and independent of the total protein concentration (Figure 6). Taken together, the com-

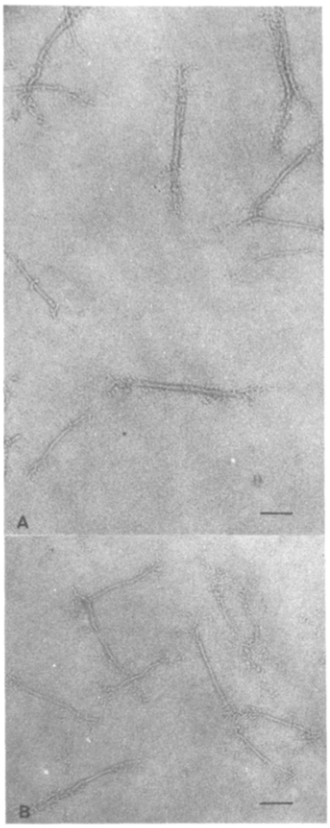

FIGURE 7: Low magnification view of cross-linked myosin minifilaments 48 h after addition of 70 mM KCl (A) and in the absence of KCl (B). The bar length corresponds to 0.1 μm.

bined evidence of electron microscopy and sedimentation studies validates the conclusion that the structures grown as a result of adding KCl to solutions of minifilaments are the filaments.

Minifilament–Filament Equilibrium. The high sensitivity of the minifilament–filament system to salt is demonstrated by examining the sedimentation profiles of myosin minifilaments 2, 23, and 48 h after addition of 70 mM KCl. Under these salt conditions, and the same protein concentration (3 mg/mL) as used before (Figure 3), a dynamic equilibrium appears to be established between the minifilaments and filaments. No significant redistribution of protein between these two structures occurs within 6 days after the initial 2-h period. This behavior is in striking contrast to the complete and relatively rapid conversion of minifilaments to filaments in the presence of 0.1 M KCl.

The minifilament–filament equilibrium established in the presence of 70 mM KCl depends strongly on the total protein concentration. At 1 mg/mL myosin the equilibrium distribution is achieved about 24 h after the addition of salt and shows only small amounts of filament species. At all protein concentrations the sedimentation profiles clearly indicate the polydispersity of the filament particles.

Low-field electron micrographs of myosin minifilaments in the absence of KCl, and 48 h after addition of 70 mM KCl, are shown in Figure 7. In the latter case, i.e., when solution conditions favor the existence of a dynamic equilibrium between minifilaments and filaments, a wide spectrum of particle sizes can be detected by centrifugation and in electron micrographs. Figure 5c presents the "continuous" size distribution of assembled myosin particles as observed after addition of 70 mM KCl to a 1 mg/mL minifilament solution. This distribution overlaps the other two distribution shown in Figure 5 of minifilaments and filaments. In agreement with sedimentation experiments (at this protein concentration), it shows the presence of relatively few fully grown filaments.

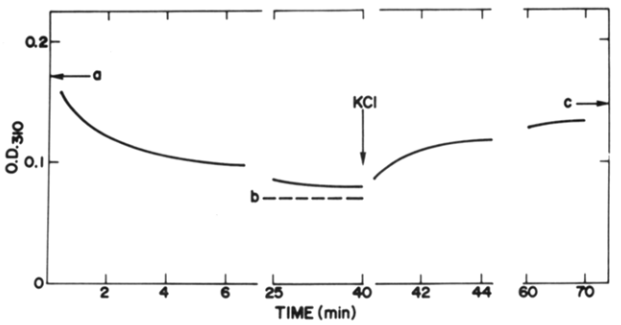

FIGURE 8: Reversibility of the minifilament to filament transition. Solution of myosin filaments (5 mg/mL) in 0.1 M KCl and 10 mM citrate–Tris was diluted 5-fold with 10 mM citrate–Tris solvent (no KCl). The time-dependent dissociation of filaments was followed by measuring the OD_{310} of this solution. At the indicated time, KCl was added to the solution (to a final concentration of 0.1 M) and the re-formation of filaments was followed. The reference turbidities (a–c) are those of separately prepared solutions. (a) Turbidity of a 1 mg/mL solution of filaments in 0.1 M KCl and 10 mM citrate–Tris (pH 8.0). (b) Turbidity of a 1 mg/mL solution of minifilaments in 10 mM citrate–Tris, pH 8.0. (c) Turbidity of a 0.9 mg/mL solution of filaments in 0.1 M KCl and 10 mM citrate–Tris buffer.

Properties of the Minifilament–Filament System. The existence of equilibrium assambly conditions (in the presence of 70 mM KCl) indicates that the minifilament to filament transition must be reversible. This should apply to all assembly conditions, even when the reaction appears to be driven in the direction of filament formation. That this is the case can be demonstrated by diluting a solution of filaments (5 mg/mL), grown in the presence of 0.1 M KCl with citrate–Tris buffer containing no KCl (Figure 8). The turbidity of such "diluted" solution decreases in a time-dependent manner and asymptotically approaches the value characteristic of minifilaments at the same protein concentrations (curve b in Figure 8). The turbidity of equally concentrated filament solution (in 0.1 M KCl) is considerably higher (curve a in Figure 8). This dissociation process can be reversed at any point by addition of salt.

Since solutions of minifilaments appear to be highly homogeneous and contain no detectable amounts of dissociated species prior to the addition of KCl, the formation of filaments must involve some type of initial dissociation of the minifilaments. The "dissociated" material can be then redistributed among the remaining minifilaments, thus initiating their growth into filaments, or can, perhaps, be utilized in a more complex manner in the formation of filaments. The initial dissociation of minifilaments, although anticipated, cannot be easily detected in the range of low salt concentrations. For example, after addition of 0.1 M KCl the turbidity of minifilament solutions increases in a millisecond time scale (as detected in a stop-flow experiment), probably masking the preceding dissociation step. However, at a higher salt concentration (0.17 M KCl), the initial dissociation of minifilaments can be demonstrated quite readily (Figure 9). The turbidity decrease depends on the protein concentration; it is pronounced below 2 mg/mL myosin and largely masked at about 3 mg/mL myosin due to the subsequent filament formation (Figure 9).

The simplest, although by no means unambiguous, test for the possible utilization of minifilaments as the nuclei of the growing filaments is the "seeding" experiment. In this test a small amount of minifilaments is added to dissociated myosin in order to induce its "explosive" polymerization. Such polymerization is characteristic of a nucleation-growth system

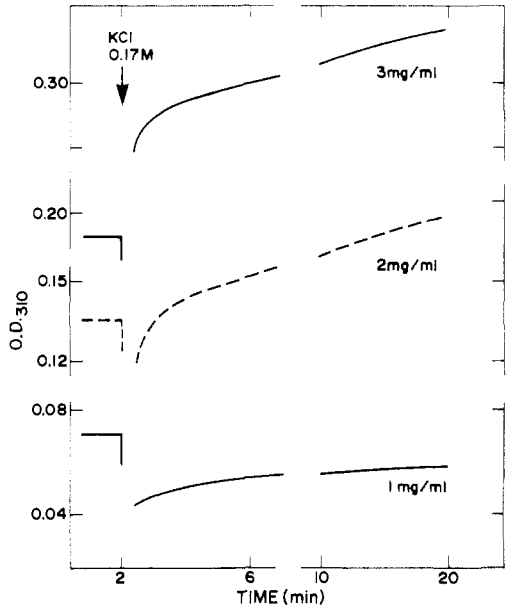

FIGURE 9: Initial dissociation of minifilaments and subsequent growth of filaments after addition of KCl to a final concentration of 0.17 M.

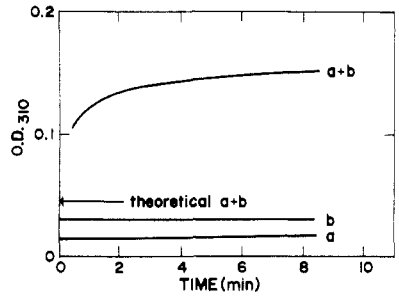

FIGURE 10: "Seeding" of filament growth with minifilaments. (a) Turbidity of minifilaments diluted into 0.17 M KCl and 25 mM Tris, pH 8.0. Protein concentration is 0.2 mg/mL. (b) Turbidity of the centrifuged myosin in 0.17 M KCl and 25 mM Tris, pH 8.0. Protein concentration is 2.0 mg/mL. (a + b) Total protein concentration is 2.0 mg/mL; 0.2 mg/mL minifilaments and 1.8 mg/mL centrifuged myosin.

which has been supplied with nuclei, In our experiments the "dissociated" myosin was obtained by separating the transparent and turbid phases that are formed after centrifugation of a filament solution (in 0.17 M KCl and 25 mM Tris, pH 8.0) at 40 000*g* for 3 h. The transparent phase has low turbidity and is referred to as the dissociated myosin. Spontaneous filament growth is detected in the solution of dissociated myosin only after the addition of a small amount of minifilaments (Figure 10, curves a + b). If a similar amount of minifilaments is diluted with a solvent, the turbidity of such solution remains low, indicating that no large structures are formed. Thus, the minifilaments indeed appear to "seed" the growth of filaments.

Discussion

One of the main aims of this work was to demonstrate that synthetic myosin filaments can be grown from solutions of minifilaments and that this process can be experimentally followed. The expectation of filament growth was based on a previous observation that addition of 0.1 M KCl to the minifilament solution resulted in an increase in its turbidity and, by implication, the formation of larger polymeric structures (Reisler et al., 1980). The morphological resemblance of minifilaments to a part of the central region in the thick filament, and the similarities between the filaments and minifilaments in their interactions with ligands (Oriol-Audit et al., 1981), indicated that minifilaments might indeed constitute a part of the filament structure rather than a polymorphic assembly form. Intuitively, one may question the stability of such a central portion of the myosin filament. However, recent studies on filament dissociation by KCl show considerable variation in the stability of the different filament regions (Trinick & Cooper, 1980; Ishiwata, 1981). They suggest, in line with our previous work (Reisler et al., 1980), the existence of different bonding interactions along the myosin filament and indicate that short bipolar myosin structures are more stable than the entire filament.

The results of sedimentation and electron microscope examinations of the structures grown from the minifilaments in the presence of 0.1 M KCl prove that these are synthetic myosin filaments. In their appearance and hydrodynamic proprerties, they resemble the synthetic filaments formed by dialyzing monomeric myosin into 0.12 M KCl (pH 8.0) solvent (Josephs & Harrington, 1966). In terms of their size distribution, they appear to be more homogeneous than the previous preparations. However, the major advantage of the new route for filament formation does not lay in the larger size homogeneity of the final assembly product. It is rather in the fact that the entire process of filament formation can be easily manipulated, brought into a convenient time scale, reversed if so desired, and hopefully separated into different reactions. As shown by our turbidity measurements, filament growth can be accomplished in minutes, hours, or days, depending on the specific protein and salt concentrations.

Of particular interest is the striking salt dependence of the minifilament–filament transition. Perhaps the simplest interpretation of this dependence can be given in terms of three interrelated dissociation–association reactions: monomer–minifilament, minifilament–filament, and monomer–filament. (The "monomer" term is used here in a strictly operational sense. It corresponds to the 6S myosin peak in the ultracentrifuge, which may also include myosin dimers.) The stability of myosin minifilaments in the presence of low KCl concentrations suggests that below 90 or 100 mM KCl the growth of filaments is rate limited by the dissociation of minifilaments. Such dissociation may be deemed necessary for addition of monomeric or dimeric myosin to the remaining undissociated minifilaments, thus initiating their growth into filaments. Since we cannot detect any minifilament dissociation (by stop-flow turbidity measurements) below 0.1 M KCl, it would follow that the growth of filaments, per se, is very rapid under such conditions and does not determine the net formation rate of the final product. Increasing the salt concentration between 50 and 130–140 mM KCl has a primary effect on increasing the rate of minifilament dissociation. This, in turn, leads to a faster net filament formation (as shown by the $t_{1/2}^{-1}$ curve in Figure 2). Increasing the salt concentration beyond 130 or 140 mM KCl destabilizes myosin filaments (under our solvent conditions) and shifts the polymerization equilibrium in favor of the dissociated species. In this salt regime, above 140 mM KCl, the net filament growth is increasingly controlled by the monomer–filament equilibrium rather than by the rate of minifilament dissociation, which presumably continues to increase. The declining filament stability or the lower intrinsic rate of filament formation (now the rate-limiting step in their net growth) can explain the decreasing rates of turbidity increase in the presence of 0.14–0.20 M KCl (Figure 2, $t_{1/2}^{-1}$ curve). This hypothetical explanation of filament growth is also consistent with the easily observed transient

minifilament dissociation in the presence of 0.17 M KCl (Figure 9).

The simple description of filament growth from minifilaments, as presented above, is by no means the only one that could fit our results, nor does it at the present stage reveal enough molecular and mechanistic details to allow rapid testing and confirmation or rejection of the assembly scheme. It is conseivable, though we consider it unlikely, that filaments do not grow directly from minifilaments but utilize for their polymerization a different, intermediate assembly form which is produced after the dissociation of minifilaments. Several lines of evidence support the simple model for the salt-induced filament growth from minifilaments: (1) Minifilaments can exist in dynamic equilibrium with myosin monomer–dimer species (Oriol-Audit et al., 1981). (2) Monomer–dimer species are present in solutions containing minifilaments and filaments, but no other intermediate structures, smaller than minifilaments, can be detected in these solutions by ultracentrifugation or electron microscope studies. (3) Under certain salt conditions (70 mM KCl), the minifilaments appear to be in dynamic equilibrium with filaments. (4) Minifilaments can be used as a seeding material to induce rapid polymerization of dissociated myosin.

The discussion of the molecular details of filament growth and the compliance of the minifilament structure with the different models for myosin filaments awaits further work. At present, for example, it is not known whether minifilament fusion occurs and possibly precedes the addition of dissociated myosin into the bipolar structure. Fraying of filaments into three subfilaments (Maw & Rowe, 1980), connected together along the central region (0.3 mM long), is suggestive of such a fusion. Future work will address and hopefully shed light on questions related to the packing of myosin in the minifilaments and the nature and sequence of events which lead to filament growth from minifilaments. The attractive features of this reaction and, most of all, the ease with which it can be monitored make the minifilament–filament system an especially promising one for the filament assembly studies.

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