MACROMOLECULAR ASSEMBLIES OF MYOSIN

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ABSTRACT The self-assembly of myosin into filamentous structures is a highly cooperative and rapid process. Nevertheless, the presence of nonequivalent bonding interactions within the filament permits differential stabilization of several macromolecular assemblies of myosin under well-controlled ionic conditions in citrate/Tris buffer at pH 8.0. We have detected and characterized bipolar myosin minifilaments, myosin octamers, and tetramers by using light scattering, analytical ultracentrifugation, and viscosity techniques. These structures have molecular weights of 8.0×10^6, 3.9×10^6, and 2.0×10^6 g/mol, sedimentation coefficients of 32S, 22S, and 18S, and radii of gyration of 990 Å, 890 Å and 790, Å, respectively. The similar radii of gyration indicate similar bipolar geometry for all these particles. The 32S minifilaments in 10 mM citrate/Tris buffer (pH 8.0) are the most stable species. The smaller 18S and 22S assemblies in 2 mM and 5 mM citrate/Tris, pH 8.0, are readily affected by low concentrations of KCl and fuse into the minifilament particles. The instability of the 18S and 22S forms of myosin assembly is also revealed by their titration with ATP. These structures are dissociated at lower ATP concentrations than the minifilaments and do not show the cooperative dissociation transitions characteristic of filaments and minifilaments. Sedimentation velocity analysis of the 18S and 22S species in the presence of ATP reveals the involvement of 10S myosin dimer in the dissociation of assembled myosin. The different forms of assembled myosin are discussed in the context of formation of myosin minifilaments.

INTRODUCTION

The in vitro self-assembly of vertebrate skeletal myosin into thick filaments was originally shown by Huxley (1963). His observations indicated that the synthetic filaments formed by decreasing the ionic strength of myosin solutions are assembled by initial antiparallel (tail-to-tail) association of myosin molecules to yield the central filament region known as the bare zone region. This step is then followed by bipolar growth of the bare zone region into long filaments, presumably by addition of monomers, parallel (head-to-tail) dimers, and other preassembled myosin units. Since then, numerous studies recently reviewed by Harrington and Rodgers (1984) and Pepe (1983) have greatly contributed to the detailed description of the effects of pH, ionic strength, protein concentration, etc., on the formation and the physical properties of synthetic myosin filaments. Thus, long before the sequence information could confirm the conclusions of solution studies (McLachlan and Karn, 1982, 1983), Josephs and Harrington (1966) emphasized the importance of ionic bonding in the assembly of myosin filaments.

The past studies of the parameters involved in myosin assembly provided the necessary background for the more recent investigations of the mechanism of filament formation. Titrations and dissociation of native myosin filaments with KCl (Trinick and Cooper, 1980; Niederman and Peters, 1982) and sodium pyrophosphate (Ishiwata, 1981), or their partial dissociation by water (Maw and Rowe, 1980), reveal the presence of different bonding interactions along the myosin filament, with its central portion exhibiting the greatest structural stability. Kinetic experiments are consistent with such distinction between the formation of the bare zone region and its growth into long filaments (Davis, 1981*a*, *b*; Higuchi and Ishiwata, 1985). Perhaps the best evidence for a two or multistep assembly of myosin and the underlying bonding differences within the filament is provided by the assembly of short, bipolar, and homogeneous minifilaments (Reisler et al., 1980). These minifilaments, which are made of 16–18 myosin molecules, and correspond to a part of the central region of filaments, can grow into regular-size filaments (Reisler et al., 1982). In this paper we report the results of recent studies aimed at clarifying the initial steps in myosin assembly. Two forms of assembled myosin, tetrameric (18S), and octameric (22S) species, are described and their properties are compared with those of myosin minifilaments.

MATERIALS AND METHODS

Myosin was prepared and its concentration determined as previously reported (Godfrey and Harrington, 1970).

Solutions of assembled myosin were prepared by a two-step dialysis procedure. Purified myosin in 0.5 *M* KCl, 10 mM phosphate buffer (pH 7.0) was clarified by centrifugation, adjusted to a concentration of 5 mg/ml, and dialyzed against 5 mM sodium pyrophosphate (PP_i) at pH 8.0. The dialyzed protein was centrifuged (30 min at 30,000 × *g*) and

redialyzed against solutions of 2 mM citrate/10 mM Tris, 5 mM citrate/20 mMTris, and 10 mM citrate/37 mMTris, all at pH 8.0 and in the absence of any additional salt.

Sedimentation velocity experiments were carried out as described earlier (Reisler et al., 1980) at rotor speeds of 20×10^3 rpm, at 20°C in a Beckman Model E analytical ultracentrifuge equipped with an ultraviolet scanner (Beckman Industries, Palo Alto, CA).

Diffusion coefficient measurements on myosin solutions in 5 mM citrate/Tris were made in synthetic boundary, double sector cells with aluminum-filled Epon centerpieces. Diffusion was carried out at 3,000 rpm in an AN-J rotor in a Beckman analytical ultracentrifuge. Protein concentrations ranged between 0.4 and 1.5 mg/ml. Although temperature was not regulated in these experiments (to improve boundary stability), the runs drifted within 2–3°C of ~25°C. Diffusion coefficients were calculated from the variance of gaussian curves describing the diffusing boundaries (Elovson et al., 1985).

Viscosity (Reisler et al., 1980) and turbidity (Reisler et al., 1982) measurements were made as before. Light scattering from myosin solutions was measured at 90° with a Farrand Model MKI fluorescence spectrophotometer (Farrand Industries, Inc., Valhalla, NY) (Reisler et al., 1980).

The angular dependence of light scattered from myosin solutions was determined as described by Jolly and Eisenberg (1976) using a modified laser autocorrelation instrument (Malvern 4300, Precision Devices and Systems Ltd., Malvern, United Kingdom) in the laboratory of Dr. H. Eisenberg at the Weizmann Institute of Science, Rehovot, Israel. All measurements were done with vertically polarized light (λ = 514.5 nm) at 20°C and covered the angular range between 12° and 150°. The scattering data were presented in the form of Zimm plots (Zimm, 1948) and were fitted to scattering curves calculated for thin rods (Eisenberg and Reisler, 1971). For further details see Eisenberg (1971).

RESULTS

Sedimentation Experiments

Sedimentation velocity experiments provide a simple and reliable test for the self-assembly of myosin, and permit a preliminary classification of the observed species. Thus, for example, myosin minifilaments show characteristic hypersharp sedimentation boundaries with $s^0_{20,w}$ = 32S (Reisler et al., 1980). The larger synthetic filaments sediment much faster ($s^0_{20,w}$ = 150S; Josephs and Harrington, 1966). Their boundaries reveal in most cases significant size distribution of the assembled particles, and at high rotor speeds these filaments dissociate into monomeric or dimeric species. The sedimentation boundaries of dissociated myosin are broadened by diffusion, and yield sedimentation constants between 6S and 10S depending on the monomer-dimer composition of protein solutions.

When solutions of myosin minifilaments prepared in 10 mM citrate/Tris (pH 8.0) or in 5 mM PP_i (pH 7.0) are dialyzed against 5 mM and 2 mM citrate/Tris, the hypersharp sedimentation profiles characteristic of minifilaments remain unchanged at both high and low protein concentrations (Fig. 1). The same profiles are also obtained by dialyzing dissociated myosin (in 5 mM PP_i, pH 8.5) against the 2 mM and 5 mM citrate/Tris solvents. These sedimentation boundaries are indicative of highly homogeneous solutions of assembled myosin. The intrinsic sedimentation coefficients of myosin in 5 mM and 2 mM citrate/Tris, and in the absence of KCl are 22.2 and 17.8S

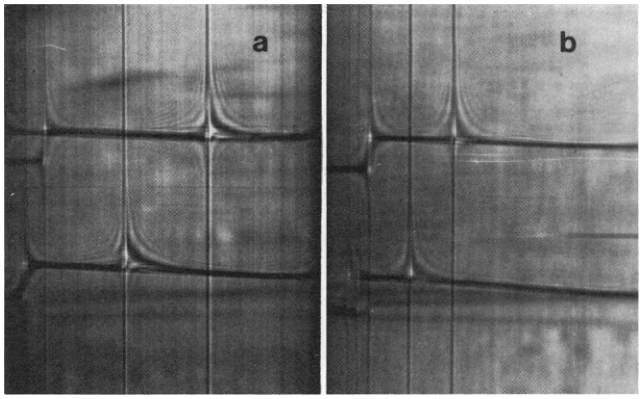

FIGURE 1 Velocity sedimentation patterns of assembled myosin. Myosin minifilaments and the 22S particles were run at a concentration of 3 mg/ml in 10 mM citrate/Tris (*a*, *upper*, wedge cell) and 5 mM citrate/Tris pH 8.0 buffer (*a*, *lower*, regular cell). The 18S particles were run in 2 mM citrate/Tris (pH 8.0) at 2 mg/ml (*b*, *upper*, wedge cell) and 3 mg/ml (*b*, *lower*, regular cell) concentrations. The sedimentation was carried out at 20°C and at rotor speeds of 20,000 rpm.

(solid curves in Fig. 2). The dashed line in Fig. 2 shows for comparative reasons the $1/s$ vs. c dependence of myosin minifilaments. We note that the sharp $1/s$ vs. c dependence is similar for the 32S minifilaments, the 22S particles, and over low protein concentrations for the 18S species as well.

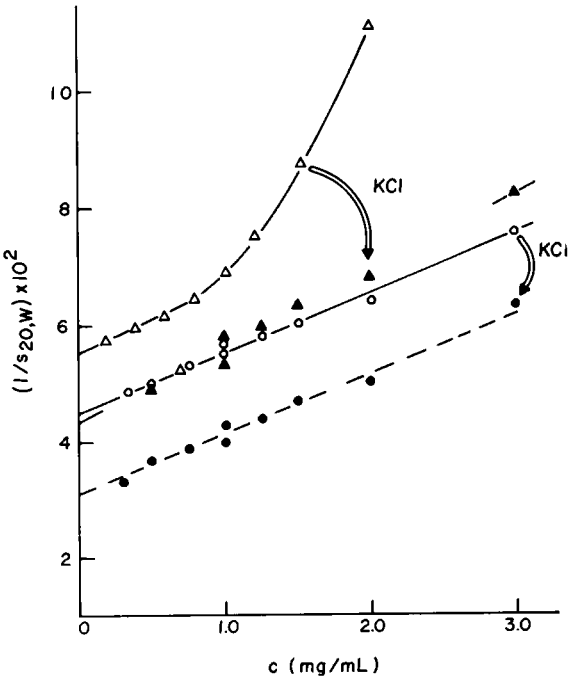

FIGURE 2 Sedimentation coefficients of the 22S (○) and 18S particles (△) in 5 mM citrate/Tris and 2 mM citrate/Tris (pH 8.0) solvents. The dashed curve shows for comparative reasons the $1/s$ vs. c dependence of myosin minifilaments in 10 mM citrate/Tris pH 8.0 (Reisler et al., 1980). The solid symbols show sedimentation coefficients of the 22S particles (●) and the 18S species (▲) after addition of 10 mM KCl to their respective solutions in 5 mM and 2 mM citrate/Tris.

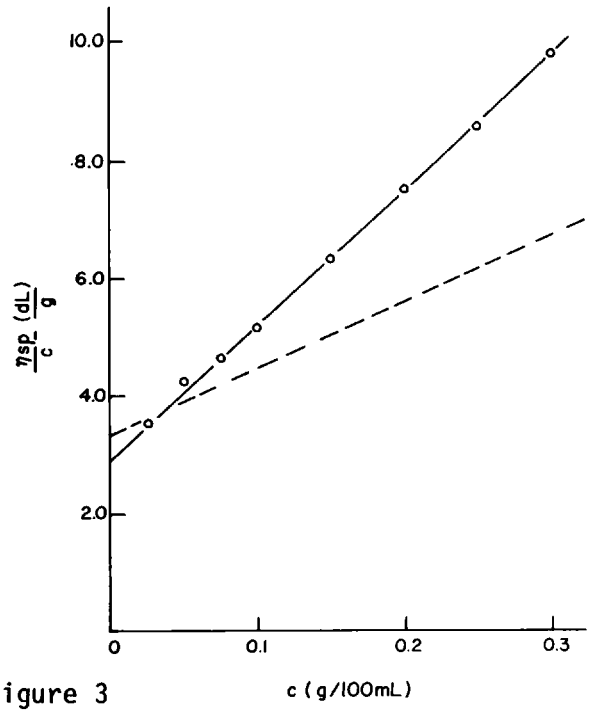

Figure 3

FIGURE 3 Reduced viscosity of 22S particles in 5 mM citrate/Tris, pH 8.0 solvent. The dashed curve shows for comparative reasons the reduced viscosities of myosin minifilaments in 10 mM citrate/Tris, pH 8.0.

TABLE I
MOLECULAR PROPERTIES OF MYOSIN ASSEMBLIES

Assembly Form	Hydrodynamic Coefficients	$M_w \times 10^{-6}$ g/mol‡	R_g, A§
Minifilaments, (10mM citrate/Tris)	$[\eta]$ = 3.42 dl/g* $s^\circ_{20,w}$ = 32.3S* $D^\circ_{20,w}$ = 0.365 × 10^{-7} cm²/s*	8.0 ± 0.4 (LS)	990 ± 80
22S (5mM citrate/ Tris)	$[\eta]$ = 3.0 dl/g $s^\circ_{20,w}$ = 22.2S $D^\circ_{20,w}$ = 0.48 × 10^{-7} cm²/s	3.85 (s, η) 4.15 (S, D) 3.9 ± 0.3 (LS)	890 ± 40
18S (2mM citrate/Tris)	$s^\circ_{20,w}$ = 17.8S	2.0 ± 0.2 (LS)	790 ± 40

*Taken from Reisler et al., (1980)
‡Molecular weights were determined from light scattering measurements (LS) presented in the form of Zimm plots (Fig. 4). Alternatively, the values of M_w were calculated from intrinsic viscosity and sedimentation data (s, $[\eta]$) by using Sheraga-Mandelkern (1953) equation, or from intrinsic sedimentation and diffusion data (s, D).
§Radii of gyration were determined from the limiting slopes of Zimm plots. Scattering curves, calculated for thin rods with the tabulated radii of gyration, fitted very well the scattering data for the 18S and 22S particles. For minifilaments, the fit to calculated curves was poorer with the optimal match obtained to thin rods with radius of gyration between 970 and 990Å.

Such $1/s$ vs. c curves are indicative of the presence of highly asymmetric polymer particles in the solutions. As discussed later, addition of small amounts of KCl (10 mM) has a profound effect on the smaller forms of assembled myosin (Fig. 2), but does not induce any detectable change in the 32S minifilaments (Reisler et al., 1982).

22S Myosin Particles

To determine the molecular size of the 22S particles we have measured the intrinsic viscosity, diffusion, and the absolute intensity of scattered light on solutions of myosin in 5 mM citrate/Tris (pH 8.0) buffer. Before taking each set of measurements, stock solutions of 22S particles were examined in the ultracentrifuge to provide a check on the reproducibility of the preparations, and the presence of any dissociated or aggregated species.

The reduced viscosities of the 22S particles are plotted in Fig. 3 against protein concentration. The extrapolated intrinsic viscosity, $[\eta]$ = 3.0 dl/g, combined with the $s^0_{20,w}$ = 22.2S (Scheraga-Mandelkern, 1953), yield the molecular weight of 3.85 × 10^6 g/mol for the 22S particles. Although the intrinsic viscosities of minifilaments and the 22S species are rather similar, the two systems differ in terms of solvent-solute interactions as reflected by their respective η_{sp}/c vs. c dependence (Fig. 3).

Diffusion coefficients of 22S particles measured in the analytical ultracentrifuge extrapolate to an intrinsic coefficient $D^0_{20,}$ = 0.48 × 10^{-7}cm²/s. The two coefficients, s and D, yield a molecular weight of 4.15 × 10^6 g/mol (Table I). Thus, the combined viscosity and sedimentation, or diffusion and sedimentation data indicate that the 22S particles are composed of 8–9 myosin molecules. In view of the likely bipolar geometry of these polymers (as discussed later), the octameric structure appears to be more plausible.

An independent determination of the molecular size of the 22S particles was made by measuring the angular dependence of light scattered from protein solution in 5 mM citrate/Tris down to an angle of 12°. The Zimm plot for the 22S material extrapolates to M_w = 3.9 ± 0.3 × 10^6 g/mol (Fig. 4), while comparable measurements of myosin minifilaments yield M_w = 8.0 × 10^6 g/mol (Table I). Thus, the light scattering results are in good agreement with the molecular weights determined by hydrodynamic methods.

The radius of gyration of the 22S particles, R_g = 890 Å, was determined from the limiting slope of the Zimm plot shown in Fig. 4. The same value was derived by fitting the angular scattering data between 12° and 90° with scattering curves calculated for thin rods. An excellent fit was obtained for rods with R_g between 890 and 900 Å. Similar analysis of scattering data collected for myosin minifilaments (R_g = 1,050 Å) produced a poorer fit to the scattering curves of thin rods. The best fit was obtained for rods with R_g between 970 and 990 Å.

The attempts to visualize the 22S and 18S particles by electron microscopy were frustrated by the instability of

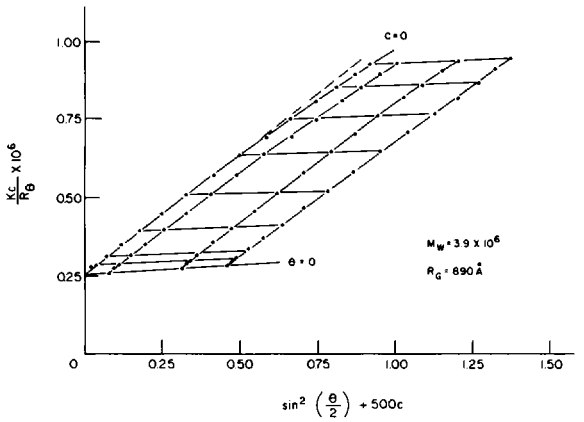

FIGURE 4 Zimm plot of total light scattering intensity from solutions of 22S particles in 5 mM citrate/Tris (pH 8.0) at 20°C. K is the optical constant, c is the protein concentration, R_θ is the reduced scattering intensity and $\theta°$ is the scattering angle. Extrapolation of this plot to $c = 0$ and $\theta = 0$ yields $1/M_w$.

these structures. If uncross-linked, they were dissociated at dilutions required for the preparation of grids. On the other hand, cross-linking reactions invariably caused rapid aggregation of the 22S and 18S particles into minifilaments or larger heterogeneous structures.

18S Myosin Particles

The anomalous biphasic $1/s$ vs. c dependence of the 18S particles in 2 mM citrate/Tris buffer (Fig. 2) indicates that all extrapolations to the intrinsic parameters of these species (at $c = 0$) need to be based on measurements carried out at low protein concentrations, up to 1 mg/ml. This requirement makes viscosity measurements impractical, and significantly decreases the precision and reliability of $s^0_{20,w}$ and $D^0_{20,w}$ determinations. Consequently, the molecular size of the 18S particles was estimated from light scattering measurements over the concentration range between 0.1 and 0.9 mg/ml. The extrapolation of the Zimm plot to $c = 0$, $\theta = 0$ (not shown here) yielded the molecular weight $M_w = 2.0 \times 10^6$ g/mol, while the radius of gyration obtained from the limiting slope of the Zimm plot was 790 Å. The experimental data could be fitted very well with scattering curves calculated for thin rods with their radius of gyration falling between 780 and 790 Å. Judged by their molecular weight, the 18S particles correspond to tetrameric myosin species.

Effect of KCl on the 18S and 22S Particles

The relative instability of myosin tetramers (18S) and octamers (22S) is most strikingly shown in Fig. 2. Addition of 10 mM KCl to solutions of 22S species leads to their rapid conversion into minifilaments. In fact, the $1/s$ vs. c curve of minifilaments is indistinguishable from that of myosin in 5 mM citrate/Tris and 10 mM KCl (Fig. 2). Moreover, the turbidity of solutions containing the 22S particles is rapidly changed to that of minifilaments after addition of 10 mM KCl.

Equally sharp transitions are noted when myosin is titrated in 2 mM citrate/Tris with KCl. In the presence of 10 mM KCl the tetrameric myosin has the turbidity and sedimentation behavior of octameric species (Fig. 2), while the presence of ~20 mM KCl imparts to the 18S material the properties of myosin minifilaments. Following these initial changes in sedimentation and turbidity of the 18S and 22S myosin solutions, additional amounts of KCl, up to a final concentration of 50 or 60 mM, have no further impact on the above parameters. At final concentrations of 70 mM KCl or more, we note a second phase of turbidity increases, reflecting the growth of synthetic filaments. The biphasic effect of KCl on the 18S and 22S particles is consistent with the formation of myosin minifilaments (between 10 and 20 mM KCl), the relative stability of minifilaments in the presence of low salt concentrations (Reisler et al., 1982), and the growth of filaments from minifilaments at higher levels of monovalent salt.

Dissociation of Assembled Myosin by ATP

Earlier work of Harrington and Himmelfarb (1972) showed that myosin and rod filaments could be cooperatively dissociated by ATP and by somewhat higher concentrations of MgATP. The destabilization of myosin polymers is due to the binding of nucleotides to the low affinity sites on the rod portion of the molecule. The highly cooperative dissociation process leads to an apparent two state monomer-polymer equilibrium (Harrington and Himmelfarb, 1972), and can easily be monitored by light scattering and analytical ultracentrifugation methods. Titrations of assembled myosin and the ensuing dissociation processes can be analyzed in terms of the Hill equation and thus can be used to probe the stability of these structures. It is assumed in such analysis that the fraction θ of dissociated myosin corresponds to the fraction of binding sites occupied by ATP (and responsible for the dissociation, Harrington and Himmelfarb, 1972). This leads to a formulation

$$\frac{[\theta]}{[1-\theta]} = \frac{[\text{sites occupied}]}{[\text{sites vacant}]} = \frac{c_{\text{monomer}}}{c_{\text{polymer}}}$$

and

$$\frac{\theta}{1-\theta} = K\,[\text{A}]^n,$$

where K is the association constant, and n is the Hill coefficient.

A representative light scattering (90°) titration of myosin minifilaments, the 22S, and the 18S particles with ATP is shown in Fig. 5. Clearly, the midpoints of dissociation are shifted toward lower ATP concentrations with decreasing concentration of citrate/Tris solvent i.e., with decreasing size of assembled myosin. This shift indicates lower

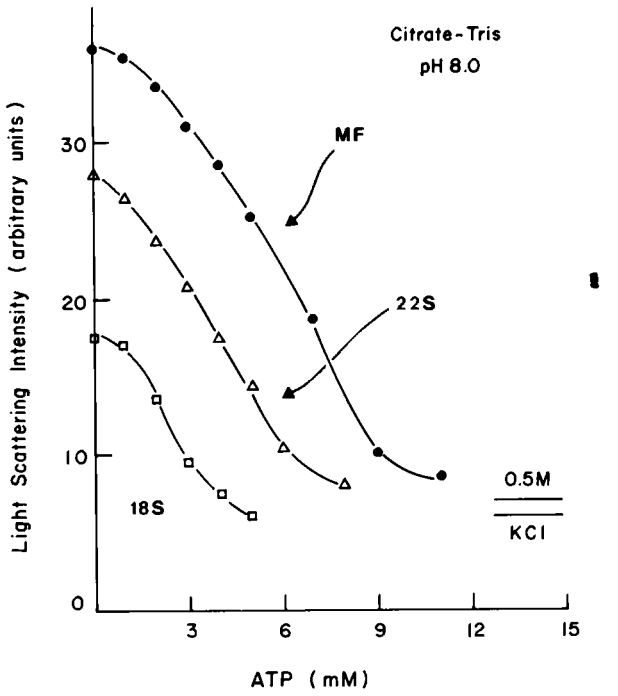

FIGURE 5 Changes in the 90° light scattering from solutions of myosin minifilaments in 10 mM citrate/Tris (●), the 22S particles in 5 mM citrate/Tris (Δ), and the 18S species in 2 mM citrate/Tris (□) as a function of added ATP. Protein concentrations were fixed at 2mg/ml.

stability of the 18S and 22S particles when compared to that of minifilaments.

By plotting the residual fraction of minifilaments (calculated from scattering data; Oriol-Audit et al., 1981) vs. nucleotide concentration, and comparing such curves with similar data obtained for myosin filaments in 0.13M KCl, 10 mM Tris (pH 8.0), we conclude that these structures,

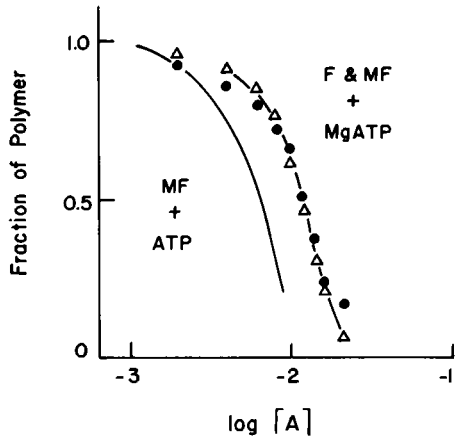

FIGURE 6 Fraction of filaments (●) and minifilaments (Δ) vs. logarithm of MgATP concentration. The dissociation profile was obtained under identical experimental conditions as those in legend to Fig. 5. The filaments were dissolved in 0.13 M KCl, 10 mM Tris (pH 8.0) and the minifilaments were in 10 mM citrate/Tris (pH 8.0). For comparison we show the profile of minifilament dissociation by ATP (solid curve without symbols).

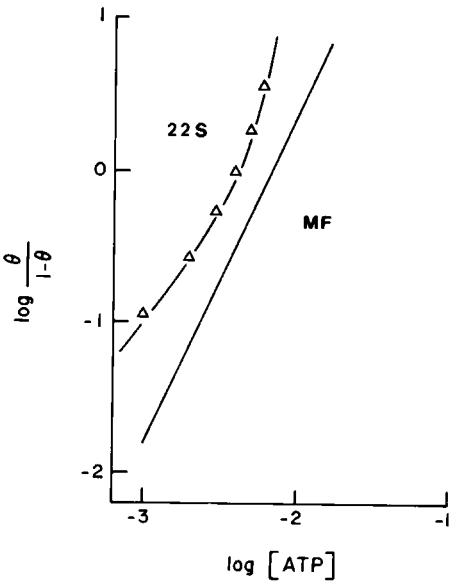

FIGURE 7 Hill plot for analysis of cooperativity of ATP binding to the 22S particles in 5 mM citrate/Tris solvent pH 8.0 (Δ). For comparison we show a similar curve obtained for myosin minifilaments (solid curve, Oriol-Audit et al., 1982).

quite different in size, show very similar stability (Fig. 6). The Hill coefficient for the MgATP-induced dissociation of minifilaments and filaments is 2.2. Similar behavior is observed when the titrations of assembled myosin are carried out with uncomplexed ATP.

The smaller 18S and 22S assemblies of myosin do not conform to the Hill analysis, and their dissociation cannot be characterized by a single Hill coefficient (Fig. 7). Inspection of the dissociation of myosin assemblies in the analytical ultracentrifuge reveals some qualitative difference between the different structures. In solutions of minifilaments the sedimentation coefficient of the polymeric material increases with increasing concentration of ATP (Fig. 8). This is consistent with decreasing concentration of minifilaments, caused by dissociation by ATP, and their strong $1/s$ vs. c dependence. Thus, judged by its sedimentation behavior, the polymeric material in these solutions indeed corresponds to minifilaments (Oriol-Audit et al., 1981). In contrast to the minifilaments, the sedimentation boundaries of the 18S and 22S species broaden, and their sedimentation coefficients decrease with increasing ATP concentrations (Fig. 8). Thus, the dissociation equilibria of small myosin assemblies are more complex than predicted by a simple two-state monomer-polymer model. Notably also, the sedimentation coefficients of dissociated myosin approach the value of 6S at high ATP concentrations, but are close to 10S at low nucleotide levels. If the 10S species correspond to myosin dimers,[1] it would appear

[1]Reisler, E., P. Cheung, N. Borochov, and J. A. Lake. Monomers, dimers, and minifilaments of vertebrate skeletal myosin in the presence of sodium pyrophosphate. Manuscript submitted for publication.

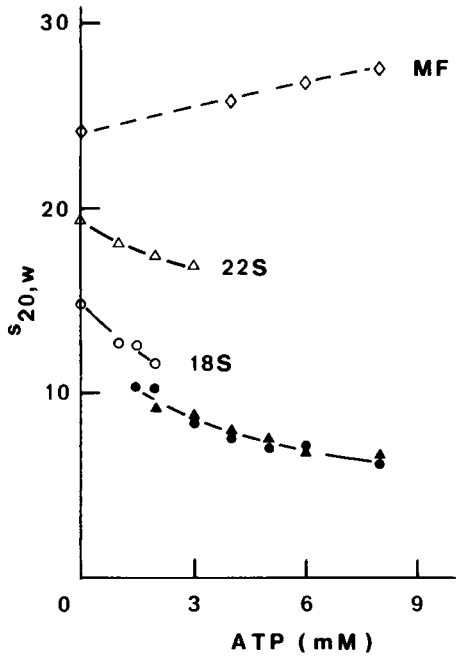

FIGURE 8 Sedimentation velocity coefficients of the polymeric (◇, △, ○) and dissociated myosin (●, ▲) obtained by titration with ATP of myosin minifilaments (◇), the 22S (△), and the 18S particles (○). All solutions were at a protein concentration of 1 mg/mL.

that the monomer-dimer equilibrium is affected by ATP as well. It is indeed very likely that the dissociation of assembled myosin involves dimeric myosin species. At least in one case, the assembly of myosin in 10 mM PP_i (pH 7.5), we were able to demonstrate a dynamic minifilament-dimer equilibrium.[1]

DISCUSSION

The main goal of this study was to investigate the initial stages of the self-assembly of myosin into minifilaments, and to identify possible precursors of this structure. The preferred route for minifilament formation involves the transfer of dissociated myosin (in 5 mM PP , pH 8.0) into 10 mM citrate/Tris buffer. In principle, quick dilutions of stock solutions of dissociated myosin into minifilament solvent could yield kinetic data, and reveal information on the individual steps of minifilament assembly. However, the entire process of minifilament formation is too fast for conventional stop-flow analysis. The alternative approach taken in this work was to simulate the medium conditions that affect the formation of minifilaments by changing the solvent composition in discrete steps. Thus, we characterized the states of myosin assembly in 2 mM and 5 mM citrate/Tris buffer.

In 5 mM citrate/Tris, we detected a homogeneous population of 22S particles, which by all molecular weight estimates should be composed of 8–9 myosin molecules. This amounts to about half the myosin content of minifilaments. The hydrodynamic parameters (s, D, η) and light scattering intensity measurements for these species showed similar dependence on protein concentration to that observed for minifilaments, and did not reveal any anomalous behavior. The smaller structures, the 18S particles in 2 mM citrate/Tris solvent, showed marked deviation from linear $1/s$ vs. c (or η_{sp}/c vs. c) dependence at protein concentrations above 1 mg/ml. Such behavior might be related to the low concentration of ions in solutions of 18S particles, and consequent primary charge effects on hydrodynamic properties of assembled myosin (Alexandrowicz and Daniel, 1963). This problem is circumvented by working at low protein concentrations, which are easily accessible to light scattering measurements. Thus, the molecular weight estimates of the 18S particles are derived from scattering experiments.

Determinations of the radius of gyration for particles of the minifilament size require a more complete and model dependent analysis of scattering curves (Eisenberg, 1971). In general, the fit of scattering data over the angular range between 12° and 90° to scattering curves calculated for thin rods was very good, and correlated well with radii of gyration obtained from limiting slopes of Zimm plots. The values of radii of gyration for the 18S, 22S, and minifilament particles (790 Å, 890 Å, and 990 Å, respectively) indicate similar geometry, and argue against major differences in myosin packing. It appears that such major packing transition occurs between dimeric and tetrameric myosin species. The radii of gyration of myosin monomers and dimers are 450 Å and 520 Å.[1] Thus, the ratios $R_{g,\,\text{minifilament}}/R_{g,\,22S}$; $R_{g,\,22S}/R_{g,\,18S}$; $R_{g,\,18S}/R_{g,\,\text{dimer}}$; $R_{g,\,\text{dimer}}/R_{g,\,\text{monomer}}$ are 1.11; 1.12; 1.52 and 1.15. In view of this evidence, we assume that, by analogy to minifilaments, the 22S and 18S particles are bipolar assemblies of myosin, and most likely correspond to octameric and tetrameric species. The existence of such particles could be easily reconciled with an antiparallel packing of parallel myosin dimer in the initial stages of myosin self assembly (Harrington and Burke, 1972; Reisler et al., 1973; Pepe, 1982; Davis et al., 1982). Unfortunately, the attempts to obtain a more direct electron microscopic evidence for the geometry of the 18S and 22S species were unsuccessful because of the instability of these particles.

The instability of the 18S and 22S particles is revealed in their titrations with both ATP and KCl. Much lower concentrations of ATP are required to dissociate these myosin polymers than are needed for minifilaments. In addition, the markedly reduced cooperativity of the dissociation reaction indicates little structural stabilization in the myosin tetramers and octamers. Similarly, the remarkably sharp effects of KCl and the rapid conversion of the small myosin assemblies into minifilaments demonstrate the inherent instability of the smaller structures. It appears then, that myosin minifilaments may represent the smallest stable form of organized myosin.

We have not found any conditions that favor a dynamic equilibrium between minifilaments and the 22S and 18S

material. If it exists, the concentrations of the smaller particles must be very low. Although it is possible to model the fusion of myosin octamers into minifilaments, and perhaps even tetramers into octamers, we have so far no direct way to monitor such transitions except for observing their end products. Consequently, we cannot properly assess the function of myosin monomers and dimers in such reactions.

That dimers might be involved in the initial assembly reactions of myosin is indicated by the presence of 10S species in ATP titrations of the 18S and 22S particles. The 10S material appears to be in equilibrium with the monomeric 6S form of myosin. We assume that the 10S species correspond to the 10S or 11S parallel myosin dimer identified in a separate work.[1] Future work will be directed at testing the importance of the small assemblies of myosin in the formation of myosin minifilaments.

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REFERENCES

Alexandrowicz, Z., and E. Daniel. 1963. Sedimentation and diffusion of polyelectrolytes. Part I. Theoretical Description. *Biopolymers.* 1:447–471.

Davis, J. S. 1981*a*. The influence of pressure on the self-assembly of the thick filament from the myosin of vertebrate skeletal muscle. *Biochem. J.* 197:301–308.

Davis, J. S. 1981*b*. Pressure jump studies on the length-regulation kinetics of the self-assembly of myosin from vertebrate skeletal muscle into thick filaments. *Biochem. J.* 197:309–314.

Davis, J. S., J. Buck, and E. P. Greene. 1982. The myosin dimer: an intermediate in the self-assembly of the thick filament of vertebrate skeletal muscle. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 140:293–297.

Eisenberg, H. 1971. Light scattering and some aspects of small angle x-ray scattering. *In* Procedures in Nuclear Acid Research. G. L. Cantoni and D. R. Davies, editors. Harper and Row Publishers, New York. 2:137–175.

Eisenberg, H., and E. Reisler. 1971. Angular dependence of scattered light, rotary frictional coefficients, and distribution of sizes of associated oligomers in solutions of bovine liver glutamate dehydrogenase. *Biopolymers.* 10:2363–2376.

Elovson, J., J. C. Jacobs, V. N. Schumaker, and D. J. Puppione. 1985. Molecular weight of apoprotein B obtained from human low-density lipoprotein (apoprotein B-PI) and from rat very low density lipoprotein (apoprotein B-PIII). *Biochemistry.* 24:1569–1578.

Godfrey, J. E., and W. F. Harrington. 1970. Self association in the myosin system at high ionic strength. I. Sensitivity of the interaction to pH and ionic environment. *Biochemistry.* 9:886–893.

Harrington, W. F., and M. Burke. 1972. Geometry of the myosin dimer in high salt media. I. Association behavior of rod segments from myosin. *Biochemistry.* 11:1448–1455.

Harrington, W. F., and S. Himmelfarb. 1972. Effect of Adenosine di- and triphosphates on the stability of synthetic myosin filaments. *Biochemistry.* 11:2945–2952.

Harrington, W. F., and M. E. Rodgers. 1984. Myosin. *Annu. Rev. Biochem.* 53:35–73.

Higuchi, H., and Ishiwata, S. 1985. Disassembly kinetics of thick filaments in rabbit skeletal muscle fibers. *Biophys. J.* 47:267–275.

Huxley, H. E. 1963. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J. Mol. Biol.* 7:281–308.

Ishiwata, S. 1981. Melting from both ends of an A-band in myofibrils. Observation with a phase contrast miscroscope. *J. Biochem.* 89:1647–1650.

Jolly, D., and H. Eisenberg. 1976. Photon correlation spectroscopy, total intensity light scattering with laser radiation and hydrodynamic studies of a well fractionated DNA sample. *Biopolymers.* 15:61–95.

Josephs, R., and W. F. Harrington. 1966. Studies on the formation and physical properties of synthetic myosin filaments. *Biochemistry.* 5:3474–3487.

Maw, M. C., and A. J. Rowe. 1980. Fraying of A-filaments into three subfilaments. *Nature (Lond.).* 286:412–414.

McLachlan, A. D., and J. Karn. 1982. Periodic charge distributions in the myosin rod amino acid sequence match cross-bridge spacing in muscle. *Nature (Lond.).* 299:226.

McLachlan, A. D., and J. Karn. 1983. Periodic features in the amino acid sequence of nematode myosin rod. *J. Mol. Biol.* 164:605–626.

Niederman, R., and L. K. Peters. 1982. Native bare zone assemblage nucleates myosin filament assembly. *J. Mol. Biol.* 161:505–517.

Oriol-Audit, C., J. A. Lake, and E. Reisler. 1981. Structural changes in synthetic myosin minifilaments and their dissociation by adenosine triphosphate and pyrophosphate. *Biochemistry.* 20:679–686.

Pepe, F. A. 1982. The structure of vertebrate skeletal-muscle myosin filaments. *In* Cell and Muscle Motility. R. M. Dowben and J. W. Shay, editors. Plenum Publishing Corp., New York. 2:141–177.

Pepe, F. A. 1983. Macromolecular assembly of myosin. *In* Muscle and Nonmuscle Motility. A. Stracher, editor. Academic Press, Inc., New York. 1:105–149.

Reisler, E., M. Burke, R. Josephs, and W. F. Harrington. 1973. Cross-linking of myosin and myosin filaments. *J. Mechanochem. Cell Motility.* 2:163–179.

Reisler, E., C. Smith, and G., Seegan. 1980. Myosin minifilaments. *J. Mol. Biol.* 143:129–145.

Reisler, E., P. Cheung, C. Oriol-Audit, and J. A. Lake. 1982. Growth of synthetic myosin filaments from myosin minifilaments. *Biochemistry.* 21:701–707.

Scheraga, H. A., and L. Mandelkarn. 1953. Consideration of the hydrodynamic properties of proteins. *J. Amer. Chem. Soc.* 75:179–184.

Trinick, J., and J. Cooper. 1980. Sequential disassembly of vertebrate muscle thick filament. *J. Mol. Biol.* 141:315–321.

Zimm, B. H. 1948. Apparatus and methods for measurement and interpretation of the angular variation of light scattering; preliminary results on polystyrene solutions. *J. Chem. Phys.* 16:1099–1116.

DISCUSSION

Discussion Chairman: Thomas D. Pollard
Scribes: Gillian Henry, John Smuda, and Ayuko Yotsukura

GERGELY: Why were the endpoints different when you added KCl to the 18S and 22S particles?

REISLER: The end product is controlled by the amount of KCl. By

addition of measured amounts of KCl, the 18S can be transformed to the 22S and subsequently to minifilaments. The stable end product is minifilaments.

VIBERT: Are there any metastable oligomers other than the tetramers, the octamers, and the minifilaments?

REISLER: As far as we can see, these are the only ones. However, we cannot preclude the existence of others; they might exist in concentrations too low to detect.

VIBERT: If these are "precursors" of the native filament, how do you relate the packing geometry of the minifilaments to the native filament?

REISLER: So far, this type of modelling agrees best with Pepe's models (see references).

VIBERT: Given that the native filament probably has three-fold rotational symmetry, is there a simple way of picturing how the minifilament relates to the native filament?

REISLER: You can envisage the minifilament as one-third of the central portion of the native filament. Splaying experiments, of the type done by Rowe and subsequently by Pepe and by ourselves, using solvents that produce minifilaments, reveal three substructures, held together only in the central region. Each one of those substructures might correspond to a minifilament. Assuming a 16-mer with eight monomers pointing in each direction, there would be 24 monomers in cross-section, one-third of such a structure. The pyrophosphate experiments were done because myosin myofibrils were shown to be dissociated by pyrophosphate treatment. We started by dissociating native filaments with pyrophosphate and ended up with minifilaments.

POLLARD: I don't understand how you can use these minifilaments to make the bare zone of a conventional filament.

REISLER: A fusion of three of the 16-mers would correspond to the central region. The transition from the octamer to the minifilament is a fusion process, but going from the minifilament to the full-size filament requires growth, a process that requires more time. We cannot follow the individual steps, and we can't tell, at this stage, whether the thickening of the filament is achieved by addition of dimers or simply by fusion. Perhaps fluorescence resonance energy transfer experiments, as performed by Anuradha Saad, will be helpful in distinguishing between fusion and simple growth.

POLLARD: If these are parts of the bare zone of a thick filament, then the heads ought to be on one side, whereas your electron micrographs seem to show the heads pointing in all directions. If this is going to be a third of the thick filament bare zone, you could imagine that it would be impossible to have the heads on the inside.

REISLER: I don't really know how much the orientation of heads in the electron micrograph corresponds to orientation in the preparation, because we normally cross-link our specimen. Depending on the extent of cross-linking, the heads are sometimes no longer visible.

STEWART: One of the ideas that Kensler presents (this volume) is that the heads of vertebrate myosin filaments (such as these) are perturbed in a number of ways. One way is their arrangement into three subfilaments within the filament. The heads have to be distorted to get to the outside. I think that this distortion of the heads is consistent with the idea that minifilaments form part of the bare zone. This could be one, although not the only, explanation of the distortion of the heads that we see.

REISLER: I agree.

VIBERT: My sense of your minifilaments is that the length of the bare zone is almost twice the length of the myosin rod minus 43 nm, whereas in native filaments, the length of the bare zone is of the order of one myosin rod, ~ 1,500-1,600 A.

REISLER: I don't have an answer to that.

SAAD: Have you made minifilaments of myosin from other sources, for example, *Nematoda*? It has been shown that there are two genetically different forms of nematode myosin. One forms the bipolar bare zone and the other, the more distal parts of the filaments.

REISLER: No, we have not.

SAAD: In an *in vitro* assembly system, your filaments are very stable entities. How do you visualize the assembly occurring? If you start off with monomers and dimers, why does the process stop at minifilaments and not go on to form filaments?

REISLER: The minifilaments are stable only under very artificial ionic strength and solvent conditions. These are not what we normally have when we monitor filament assembly or those that exist in the cell. Pyrophosphate minifilaments are much more easily perturbed. They can be isolated under stable conditions, but further growth can easily be induced.

SAAD: Do you think a *Nematode*-like system can exist in vertebrates?

REISLER: There is no way of telling. We do not see any difference between myosin that forms minifilaments and myosin that does not, but we have not looked at the sequence of these myosins or at microheterogeneity.

SAAD: C-protein and M-protein bind only to the center. It would be interesting to see if there are two different types of myosin.

POLLARD: There is some information about two different types of myosin in chicken skeletal muscle, based on unpublished antibody work done by Albert Wong in my lab. He made antibodies against human platelet myosin and a subset of these antibodies reacted with all species tested. When he stained skeletal muscle with these antibodies, he found that they only stained the center of the thick filament.

REISLER: Perhaps we should add the report of Niederman and Peters in which they isolate what they call "bare zone assemblages". This could be an indication of two types of myosin.

LEVINE: Returning to *Nematode* myosin, H. Epstein has some pictures showing assembly of full thick filaments of both myosin types.

REISLER: I had the impression that both chicken isozymes form full thick filaments.