Intermolecular Versus Intramolecular Interactions of *Dictyostelium* Myosin: Possible Regulation by Heavy Chain Phosphorylation

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Abstract. Dictyostelium myosin has been examined under conditions that reveal intramolecular and intermolecular interactions that may be important in the process of assembly and its regulation. Rotary shadowed myosin molecules exhibit primarily two configurations under these conditions: straight parallel dimers and folded monomers. All of the monomers bend in a specific region of the 1860-Å-long tail that is 1200 Å from the head–tail junction. Molecules in parallel dimers are staggered by 140 Å, which is a periodicity in the packing of myosin molecules originally observed in native thick filaments of muscle. The most common region for interaction in the dimers is a seg-ment of the tail about 200-Å-long, extending from 900 to 1100 Å from the head–tail junction. Parallel dimers form tetramers by way of antiparallel interactions in their tail regions with overlaps in multiples of 140 Å.

The folded configuration of the myosin molecules is promoted by phosphorylation of the heavy chain by *Dictyostelium* myosin heavy chain kinase. It appears that the bent monomers are excluded from filaments formed upon addition of salt while the dimeric molecules assemble. These results may provide the structural basis for primary steps in myosin filament assembly and its regulation by heavy chain phosphorylation.

IN nonmuscle cells, genetic experiments have proven that myosin is critical for cytokinesis and important for efficient cell migration (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987; Manstein et al., 1989). Genetic experiments have also established that the tail portion of myosin, which is involved in filament assembly, is essential for myosin function in vivo (De Lozanne and Spudich, 1987). Myosin filaments appear to be transient structures that organize and relocate in response to internal and external stimuli (Yumura and Fukui, 1985). The process of filament assembly requires precise control that appears to be accomplished by either phosphorylation of the myosin light chain (Smith et al., 1983; Susuki et al., 1978) or by phosphorylation of the heavy chain (Kuczmarski and Spudich, 1980; Takahashi et al., 1983; Cote and McCrea, 1987).

Dictyostelium myosin exists as thick filaments in vivo, and these thick filaments translocate to the cortex in response to the chemoattractant cAMP (Yumura and Fukui, 1985). This translocation is correlated with in vivo phosphorylations of the myosin molecule (Berlot et al., 1985, 1987; Nachmias et al., 1989). Phosphorylation of the myosin heavy chain in vitro inhibits thick filament formation (Kuczmarski and Spudich, 1980; Cote and McCrea, 1987; Ravid and Spudich, 1989).

The assembly state of myosin in vitro is highly dependent on ionic interactions (Josephs and Harrington, 1966). Myosin thick filaments disassemble into monomers at high ionic strength. This property has been used to visualize the basic structure of myosin, but functional, physiologically significant conformations of the molecule may be difficult to resolve in high salt. The tail of *Dictyostelium* myosin, like that from all other organisms, contains alternate clusters of positive and negative charges that may participate in intramolecular and intermolecular electrostatic interactions (McLachlan and Karn, 1982; Warrick and Spudich, 1987). These contacts may be inhibited by shielding of charges in a high ionic strength solution. At physiological ionic strength myosin is primarily filamentous. As the ionic strength is lowered, filaments begin to disassemble revealing important configurations of myosin, some of which can be affected by phosphorylation (Kuczmarski et al., 1987, 1988; Reisler et al., 1986; Trybus and Lowey, 1984, 1987). In the work of Kuczmarski et al. (1987), *Dictyostelium* myosin at low ionic strength was shown to disassemble into monomers, parallel dimers, and antiparallel tetramers. In molluscan catch muscle myosin, tail phosphorylation favors a folded configuration (Castellani and Cohen, 1987). Phosphorylation of the heavy chain appears to have an opposite effect on the conformation of *Physarum* myosin (Takahashi et al., 1983).

In this paper, we have explored the intermolecular contacts in dimers and tetramers, the formation of a bent monomeric

configuration of *Dictyostelium* myosin, and the role of heavy chain phosphorylation in regulation of filament assembly.

were excised from the gel and counted in a scintillation counter (LS7500; Beckman Instruments Inc., Palo Alto, CA).

Materials and Methods

Proteins

RNA-free myosin was purified from *Dictyostelium* cells strain Ax3 as described (Griffith et al., 1987). Myosin was stored in a high salt buffer (myosin storage buffer: 0.5 M KCl, 1 mM DTT, 1 mM EDTA, 10 mM Tris, pH 7.5) on ice at a concentration of about 3 mg/ml. The protein also was stored for up to 4 mo in the above buffer diluted 1:1 with glycerol at −20°C. *Dictyostelium* myosin heavy chain kinase was partially purified from a crude membrane fraction of developed cells. *Dictyostelium* cells were developed as described by Berlot et al. (1985) and disrupted by sonication; the crude membrane fraction was sedimented at 30,000 g. This membrane fraction was extracted in 50 mM Tris, pH 7.5, 30% sucrose, and 20 mM pyrophosphate, and then centrifuged at 100,000 g. The supernatant was sequentially chromatographed on phenyl-Sepharose CL-4B and Affi-Gel blue, and then gel filtered on Superose 12, resulting in a 200-fold purification. Fab' fragments of a monoclonal antiDictyostelium myosin antibody, referred to as My1, had been prepared by Peltz et al. (1985) and stored at −80°C. Rabbit skeletal muscle heavy meromyosin (HMM)[1] was a gift from Dr. R. Cooke and prepared as described (Weeds and Taylor, 1975).

Sample Preparation and EM

Dictyostelium myosin samples (1–2 mg/ml) were dialyzed overnight into a low ionic strength buffer (10 mM Tris, 1 mM DTT, 1 mM EDTA, pH 7.5) at 4°C. In experiments where filaments were formed, dialyzed samples were mixed 1:1 with a similar buffer containing twice the final salt concentration desired (30 mM or 60 mM KCl) for 30 min at 22°C to allow assembly. In experiments where myosin molecules were labeled with monoclonal antibody My1, the antibody–myosin mixture was incubated for 2–3 h at a 1:1 molar ratio on ice in myosin storage buffer (Flicker et al., 1985). For rotary shadowing, the samples were diluted to 30–50 μg/ml (80 μg/ml in the filament preparations) myosin in 50–60% glycerol in their respective buffers. The samples were sprayed immediately onto freshly cleaved mica and prepared as described (Flicker et al., 1985).

Morphometry of electron micrographs of rotary shadowed myosin was done using a microcomputer tracking system (Hynes et al., 1987). For this purpose, 8 × 10 prints at several magnifications were imaged with a video camera. The lengths of segments of the tail were measured by tracing the images on the video screen with the digitizing cursor. For calibration, an electron micrograph of a tropomyosin paracrystal taken at the same magnification and printed identically to the images of myosin was used. The x and y coordinates of the digitizing system were calibrated by counting 10 or more of the 395-Å periods of the paracrystal in each direction. All values for the length measurements are given with their standard deviations. Numbers of bent monomers and parallel dimers of myosin were counted either on prints or negatives.

Phosphorylation of Myosin

In a typical reaction, 25 μg of myosin were incubated with 50 μg of the kinase fraction in 310 μl of buffer (12.5 mM KCl, 10 mM Tris, 1 mM DTT, 3 mM MgCl$_2$, 0.2 mM ATP, pH 7.5) on ice, or at 22°C. Aliquots of 50 μl were withdrawn at specific time points and the reaction stopped by addition of 20 μl of 1 ml hexokinase and glucose buffer (0.1 M glucose, 50 U/ml hexokinase (14 U/mg; type III; Sigma Chemical Co., St. Louis, MO), 10 mM Tris, 1 mM DTT, pH 7.5) to deplete ATP. The samples were incubated 10 min at 22°C and then dialyzed in the low salt buffer on ice and prepared for EM. To determine ^{32}P incorporation into myosin, the same experiment was carried out in parallel using γ-^{32}P-labeled ATP (550 cpm/pmol), and the reaction was stopped by the addition of 10% TCA. The phosphorylated myosin was sedimented in a microfuge after 15 min at 0°C, resuspended in 20 μl SDS sample buffer, and electrophoresed on 7.5% SDS polyacrylamide gels. After staining with Coomassie-blue (Laemmli, 1970), gels were scanned with an RFT scanning densitometer (Transidyne General Corp., Ann Arbor, MI) at 600 nm, and the peaks were cut out and weighed. These weights were converted to protein concentrations using rabbit skeletal myosin as a standard. To determine incorporation of ^{32}P, the myosin bands

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1. *Abbreviations used in this paper*: HMM, heavy meromyosin.

Results

Myosin Configurations in Low Ionic Strength

Dictyostelium myosin filaments disassemble in very low ionic strength buffer (Kuczmarski et al., 1987). Rotary shadowed myosin molecules exhibit primarily two configurations under this condition; straight parallel dimers and bent monomers (Figs. 1 and 2). The bend in the monomers is 1200 ± 30 Å (n = 60) from the head–tail junction. The total length of the tail is 1860 ± 30 Å. The bent tail folds back on itself such that the final 660 Å of the tail contacts the region of the tail proximal to it. In typical myosin preparations, 90–94% of the molecules are in parallel dimers and 6–10% are folded monomers (5 myosin preparations; n = 1000). Some dimers further interact to form antiparallel and parallel tetramers. These configurations also are found, along with thick filaments, in buffers with salt concentrations up to 60 mM KCl and 2 mM MgCl$_2$. This ionic strength is close to physiological for *Dictyostelium* (J. D. Pardee, personal communication).

Staggering of the Myosin Molecules in the Dimer

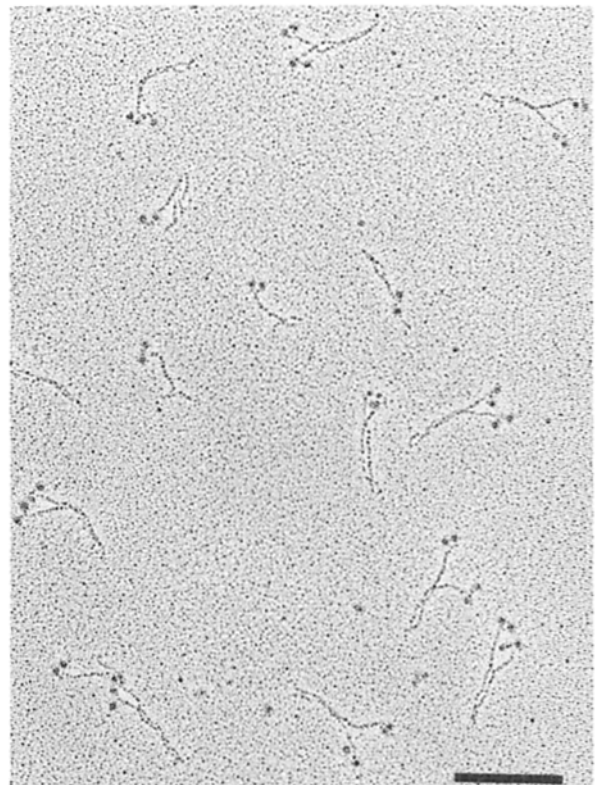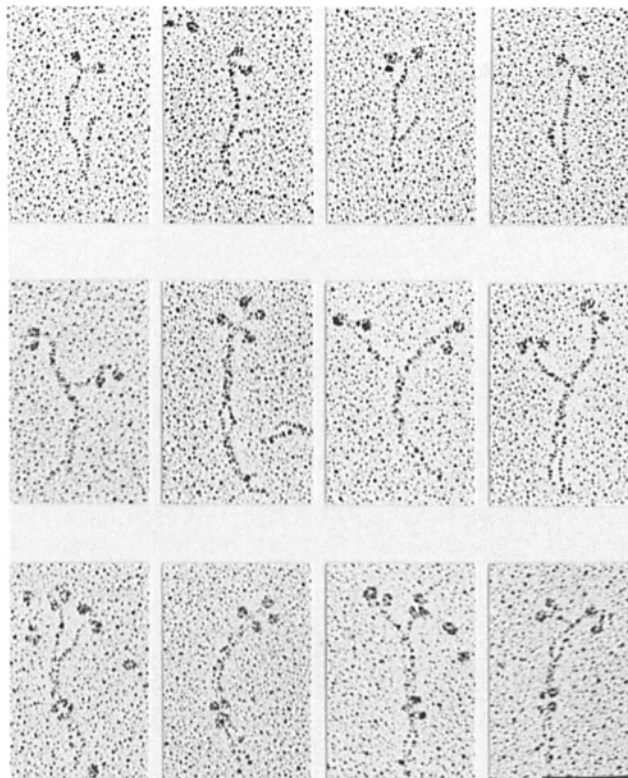In dimers the myosin molecules interact in parallel along

Figure 1. Parallel dimers and bent monomers are seen in an image of rotary shadowed *Dictyostelium* myosin molecules under conditions of very low ionic strength (10 mM Tris, 1 mM DTT, 1 mM EDTA, pH 7.5). Bar, 0.2 μm.

Figure 2. Selected rotary shadowed *Dictyostelium* myosin molecules in very low ionic strength. (*Top row*) the bend in the monomers is at a site 1,200 Å from the head–tail junction. (*Middle row*) the molecules in parallel dimers are staggered by 140 Å. The extent of contact between the tails is variable. (*Bottom row*) monoclonal Fab' fragments bound to myosin dimers highlight the stagger between the myosin molecules. An Fab' often binds to each heavy chain so that two pairs of Fab's can be distinguished on a dimer, separated by a distance of ∿160 Å. Bar, 0.1 μm.

their tail portions. The contact region varies in length usually including the center third of the tail (Table I; Figs. 2–4). However, the two molecules in a dimer are not directly aligned with each other but instead are staggered. Despite differences in the length of the contact between molecules, the stagger between them is a constant 140 Å in all dimers (Table II; Fig. 2).

A schematic drawing of a dimer with definition of the distances measured is shown in Fig. 3. The staggering in each dimer was measured in two ways: (*a*) as the difference in the lengths HT1 and HT2, and (*b*) as the difference in the lengths

Table I. Average Length of Segments of the Myosin Tail

Segment	Length ± SD Å (*n*)
HT1	660 ± 220 (102)
HT2	500 ± 230 (105)
TE1	340 ± 220 (72)
TE2	500 ± 220 (71)
TT	940 ± 350 (68)

The segments are defined in Fig. 3. *n* is the number of molecules measured. Each individual measurement was determined to a resolution of ∿30 Å. The SDs demonstrate the broad distribution of the lengths of the contact region in the dimer population.

Table II. Stagger Distance in Myosin Dimers

Distance	Length ± SD Å (*n*)
Δ HT	140 ± 30 (110)
Δ TE	140 ± 30 (76)
Δ Fab'	160 ± 20 (11)

Δ HT = HT1 − HT2 (Fig. 3); Δ TE = TE2 − TE1 (Fig. 3); Δ Fab' = distance between the two pairs of Fab's (Fig. 2).

TE2 and TE1. The staggering also is demonstrated strikingly by the staggered binding of Fab' fragments of a monoclonal antibody against *Dictyostelium* myosin (Fig. 2, *bottom row*). This antibody My1, was shown previously to bind to the myosin tail ∿1,200 Å from the head–tail junction (Flicker et al., 1985). One Fab' binds to each heavy chain so that two separated pairs of Fab's can be distinguished on a dimer. The distance between the two pairs of Fab' fragments is 160 ± 20 Å, in good agreement with the values obtained by direct measurements of the stagger of the myosin molecules (Table II; Fig. 2).

Identification of the Tail Segment Important for Dimerization

The region critical for interaction between the tails in dimers was mapped by determining the contacting segments for each molecule in 68 dimers to find the shortest segment in contact common to all the molecules. The molecule with the longer HT region was defined as molecule 1 of the pair (Fig. 3). In Fig. 4 (*top*) the regions in contact along the myosin tail for molecules 1 are drawn on the myosin tail coordinates. All the molecules in dimers interact with the adjacent molecule within a region of ∿350 Å (*shaded zone*). This segment extends from 900–1,250 Å in molecules 1 (Fig. 4, *top*) and from 750–1,100 Å in molecules 2 (not shown). In Fig. 4 (*center*) the number of molecules interacting within each 50-Å interval along the tail is plotted for molecules 1 and 2. In this representation, >85% of molecules 1 (*solid line*) show contact in the region of 900–1,250 Å (*shaded zone*) from the head–tail junction. Similarly, >85% of molecules 2 (*broken line*) show contact in the region of 750–1,100 Å (*cross-hatched zone*) from the head–tail junction. These two zones are shifted such that when they interact with one another, as shown in
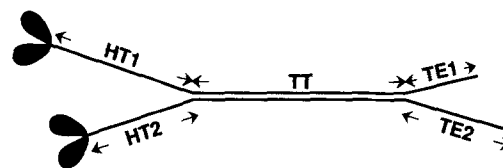
Figure 3. Schematic drawing to scale of a staggered myosin dimer. The stagger between myosin molecules in dimers and the portions of the myosin tail apparently in contact in dimers was determined from the lengths of the following segments on each molecule: *HT*, the distance from the head–tail junction to the point of contact with the adjacent molecule; *TE*, the distance from the last point of contact between the tails to the end of the tail, and *TT*, the length of the contact region between the two molecules in a dimer. The molecule with the longer *HT* region was defined as molecule 1 of the pair.
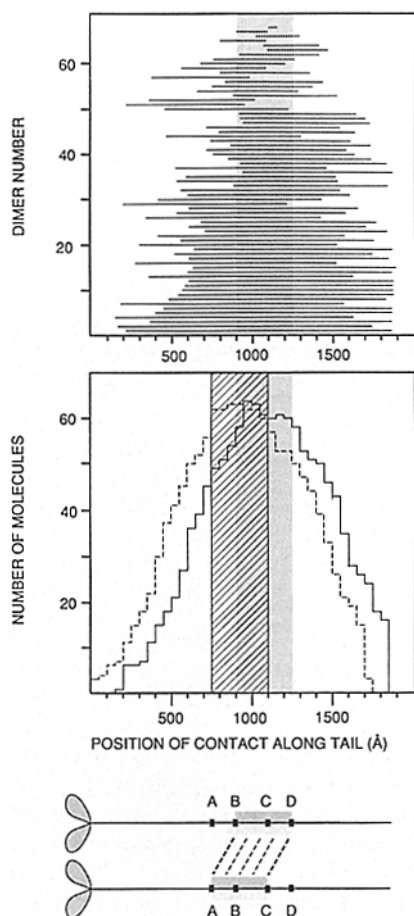
Figure 4. (*Top*) the regions of contact for molecules 1 (defined in legend to Fig. 3) were drawn on the coordinates of the myosin tail for 68 dimers. Although the length of contact is variable, each molecule interacts with its respective adjacent molecule in the dimer within a segment of ∼350 Å (*shaded zone*). This segment extends from 900 to 1,250 Å for molecules 1. (*Middle*) the number of molecules interacting within each 50 Å interval along the tail is plotted for molecules 1 (*solid line*) and molecules 2 (*broken line*). The stagger between molecules 1 and 2 is clearly shown as a shift between the two histograms. More than 85% of molecules 1 show contact in the region 900–1,250 Å (*shaded zone*) from the head–tail junction. More than 85% of molecules 2 show contact in the region 750–1,100 Å (*cross-hatched zone*) from the head–tail junction. (*Bottom*) a schematic drawing of two myosin molecules showing a possible interaction between them that would establish a dimer with a 140-Å stagger. Each myosin molecule has the potential to be either molecule 1 or molecule 2 in a dimer. The region *AD* marks the entire portion of myosin tail, which has a high probability of being in contact in a dimer. The segment *AC* corresponds to the cross-hatched portion of the histogram above, where molecules 2 are commonly in contact. The segment *BD* corresponds to the shaded region in the diagrams above, where molecules 1 generally are in contact. The common segment *BC* has the highest probability of being in contact regardless of the position of the molecule in the dimer. Interaction between the segment *BD* on one myosin molecule and the segment *AC* on a second myosin molecule would establish a parallel dimer staggered by 140 Å.

Fig. 4 (*bottom*), the molecules become staggered in the dimer by 145 Å. Note that the region of overlap of these two zones (Fig. 4, segment *BC, bottom*), 900–1,100 Å, has the highest

probability of being in contact regardless of the position of the molecule in the dimer.

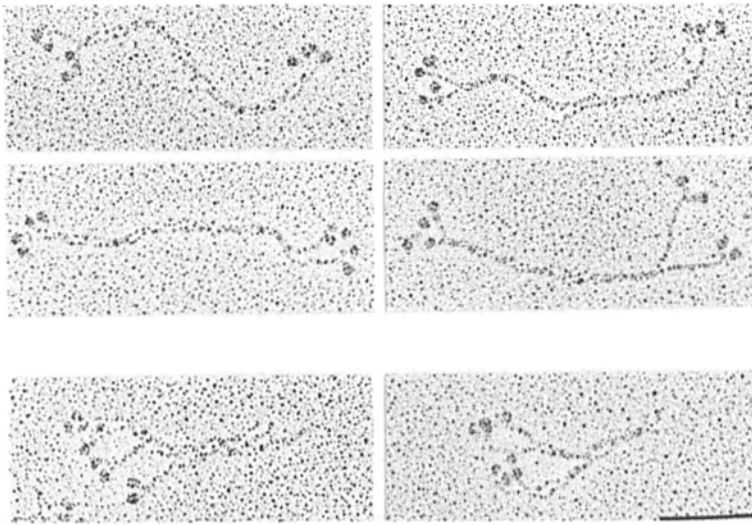### Antiparallel and Parallel Interactions among Dimers

In addition to monomers and dimers, antiparallel and parallel tetramers are seen at low ionic strength (Fig. 5). We measured the length of the overlap in antiparallel tetramers directly or by measuring the distance between the head–tail junctions of antiparallel molecules and subtracting it from 2 × 1860 Å, the total length of two tails. The overlaps center around two values both multiples of 140 Å; 420 ± 20 Å (3 × 140 Å); and 300 ± 30 Å (2 × 140 Å). Out of 34 tetramers, 70% were of the 3 × 140 Å type and 30% were of the 2 × 140 Å type.

Schematic representations of possible antiparallel interactions within tetramers are shown in Fig. 6. A single 140-Å antiparallel overlap would be established solely by interaction between the terminal 140-Å regions of opposing dimers. This arrangement was not seen experimentally. An overlap of 2 × 140 Å allows a second type of contact, one between the terminal 140-Å segment of one myosin molecule, and the penultimate 140-Å segment of an antiparallel molecule (Fig. 6, *solid triangles*). If we assume that this second type of interaction is critical for formation of antiparallel tetramers, the 3 × 140 Å overlap would be most prevalent since up to four interactions of this type are possible in this configuration (Fig. 6). The 2 × 140 Å overlap species would be less frequent since only two interactions of this type are possible. This predicted pattern is consistent with the observations described above.

Parallel tetramers are rare at very low ionic strength. However, in the eight that we detected, the two dimers are staggered by ∼300 Å (Fig. 5), such that each molecule is staggered by 140 Å relative to its neighbors. The number of tetramers and larger aggregates increases in the presence of 5–10 mM KCl (data not shown).

Phosphorylation of the Heavy Chain Correlates with Bending of the Myosin Tail

To test the possibility that heavy chain phosphorylation causes bending of the tail, we used a partially purified heavy chain kinase from developed *Dictyostelium* cells to phosphorylate myosin (Ravid and Spudich, 1989). Phosphorylation by this kinase increases the number of bent monomers visualized by rotary shadowing, both in low ionic strength buffer and at higher salt concentrations. The bend is 1,200 ± 30 Å (*n* = 40) from the head–tail junction as seen in monomers before additional phosphorylation in vitro. The percentage of myosin bent monomers is proportional to the amount of heavy chain phosphorylation as shown in Fig. 7. These results are accumulated from four experiments using four kinase preparations and myosin from two preparations. Myosin was incubated with the kinase for times ranging from 20 to 120 min at temperatures of 0°C or 22°C. Control experiments without kinase or without ATP showed neither phosphorylation nor an increase in the number of bent monomers. The distribution of phosphates among the heavy chains is not homogeneous since only 43% of the molecules are bent monomers when 1 mol of ^{32}P is incorporated per mole of myosin heavy chain. Several sites on the heavy chain may

be phosphorylated (Vaillancourt et al., 1988; Ravid and Spudich, 1989).

Bent Monomers Are Excluded from Thick Filaments

Dictyostelium myosin forms thick filaments and becomes insoluble at salt concentrations between 25 and 80 mM (Kuczmarski and Spudich, 1980; Ravid and Spudich, 1989). Homogeneity and size of myosin filaments formed in vitro are dependent on the precise methods and conditions of pH and cation concentrations used to prepare them (Huxley, 1963; Kuczmarski et al., 1987). The thick filaments we observed are variable in size and appearance. Whereas most filaments appear bipolar, in some cases the bare zone is not clearly discernable (Fig. 8).
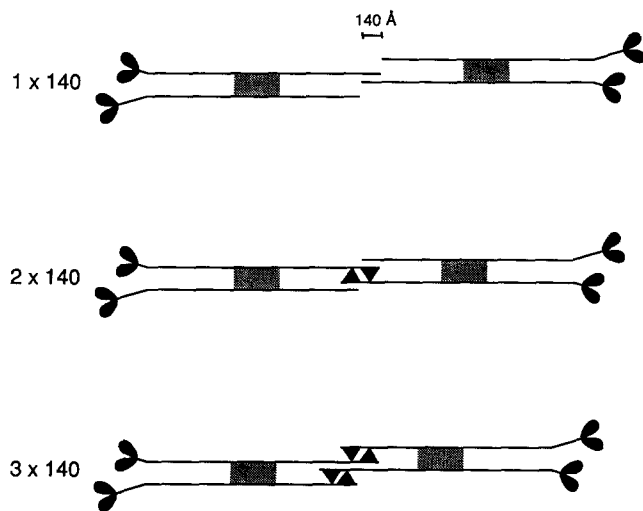

Figure 6. Schematic representation to scale of antiparallel tetramers showing possible interactions between overlapping segments. Contacts between the terminal 140-Å segment of one myosin molecule and the penultimate 140-Å segment of an antiparallel myosin molecule are shown by the solid triangles. Note that this type of contact does not occur in the case of a 1 × 140-Å overlap, and such tetramers are not seen experimentally.

Monomers and dimers coexist with filaments observed at 30 mM KCl and 60 mM KCl (Fig. 8). The number of dimers is low, but the number of bent monomers appears similar to that seen before KCl was added, as if the bent monomers are excluded from thick filaments. To quantitate our observations, we counted the monomers and dimers visible at different salt concentrations relative to the number of skeletal muscle HMM molecules included as a standard (HMM does not polymerize and does not appear to intercalate in the thick filaments). We found that the ratio of the number of bent monomers to the number of HMM molecules is nearly constant in this range of salt concentrations, so that at least 90% of the bent monomers are excluded from thick filaments (Fig. 9). The ratio of the number of parallel dimers to the number of HMM molecules changed dramatically by a factor of >40 when the KCl concentration was increased from 0 to 60 mM, consistent with the parallel dimer being the basic unit for thick filament assembly. In experiments with phosphorylated myosin the number of bent monomers was higher in the low salt buffer (Fig. 7) and proportionally higher when the salt concentration was increased and dimers assembled. Starting with a control sample with 6% bent monomers in low salt, they accounted for 34% of the unassembled molecules in a buffer with 37 mM KCl. After incorporation of 0.33 mole of ^{32}P/mole of heavy chain, 22% of the molecules were bent in low salt and they accounted for 48% of the unassembled molecules at 37 mM KCl. In a sample where 43% of the molecules were bent monomers in low salt, in 37 mM KCl the bent configuration accounted for 75% of the unassembled molecules. These experiments indicate that additional bent monomers, formed by in vitro phosphorylation, are also excluded from filaments formed in a buffer containing 37 mM KCl.

Discussion

We propose that heavy chain phosphorylation regulates myosin assembly by sequestering the molecules in a bent monomeric conformation that is unable to incorporate into dimers or thick filaments. As noted above, 6–10% of the myosin molecules in a typical preparation are in a bent monomeric
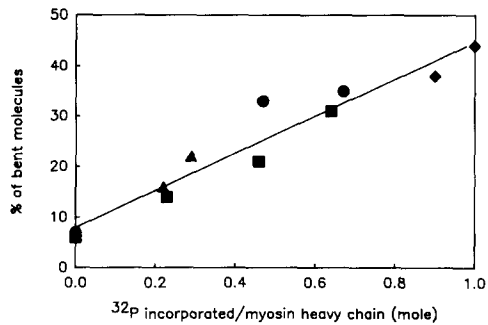
Figure 7. Dependence of the percentage of bent myosin monomers on the degree of phosphorylation of the heavy chain. The plot summarizes results of four experiments using four different kinase preparations and myosin from two preparations. 6–7% of the myosin molecules were bent before the kinase treatment.

configuration at low ionic strength. Similarly, <10% of the maximal phosphorylation level is found in a typical myosin preparation. About 0.25 mol of phosphate is detected per mole of myosin heavy chain isolated from vegetative *Dictyostelium* cells grown in [^{32}P]orthophosphate (Kuczmarski and Spudich, 1980), whereas 4 mol of phosphate/mol of myosin has been achieved in vitro (Ravid and Spudich, 1989). The same type of bent monomers are formed in *Dictyostelium* myosin concomitant with in vitro heavy chain phosphorylation; the tail bends at a specific site 1,200 Å from the head–tail junction. We further reported here that the bent monomers are excluded from thick filaments. Myosin that has been phosphorylated in vitro to the level of 4 mol of phosphate/mol of myosin heavy chain is soluble from 0 to 250 mM salt, demonstrating that this heavy chain phosphorylation inhibits myosin assembly (Ravid and Spudich, 1989).

A possible relationship between the parallel dimer and bent monomer is depicted in Fig. 10. The dimer may be stabilized by multiple electrostatic interactions along a specific

region of the tail (Fig. 4). In the monomeric folded configuration, the same region interacts with the tail segment adjacent to it. This adjacent segment contains several in vitro phosphorylation sites. Phosphorylation at these sites may provide the sufficient energy change that favors the bent monomer form. Stabilization of the bent monomer may result from favorable interactions between the phosphorylated residues and positively charged groups in the region important for parallel dimer formation. The tail of *Dictyostelium* myosin is phosphorylated in vivo (Peltz et al., 1981), but the sites of phosphorylation are not known. Several in vitro phosphorylation sites have been localized to the COOH-terminal part of the tail, starting 1,400 Å from the head–tail junction as shown schematically in Fig. 10 (Pagh et al., 1984; Kuczmarski et al., 1988; O'Halloran et al., manuscript submitted for publication; Vaillancourt et al., 1988).

In this study, we have analyzed the properties of the parallel dimer. A 140-Å periodicity in myosin thick filaments was first described in striated muscle (Huxley and Brown, 1967). This periodicity has been seen in *Dictyostelium* myosin synthetic filaments (Stewart and Spudich, 1979; Pagh and Gerisch, 1986) and in paracrystals formed from expressed tail fragments (De Lozanne et al., 1987; O'Halloran et al., manuscript submitted for publication). Our observations show that this primary structural feature of the thick filament is expressed in the *Dictyostelium* myosin dimer. Staggered parallel dimers have been described previously for other types of myosin. In those cases, the staggers were multiples of 140 Å (Davis et al., 1982; Trybus and Lowey, 1987; Wijmenga et al., 1987).

The pattern of charged residues in the tail of *Dictyostelium* myosin indicates that strong electrostatic attractions and repulsions result when two molecules are placed side by side (Warrick et al., 1986). Analysis of these interactions, first carried out for *C. elegans* myosin (McLachlan and Karn, 1982, 1983), predicts that the strongest attraction would be at a stagger of 98 residues or 145 Å (Warrick et al., 1986;
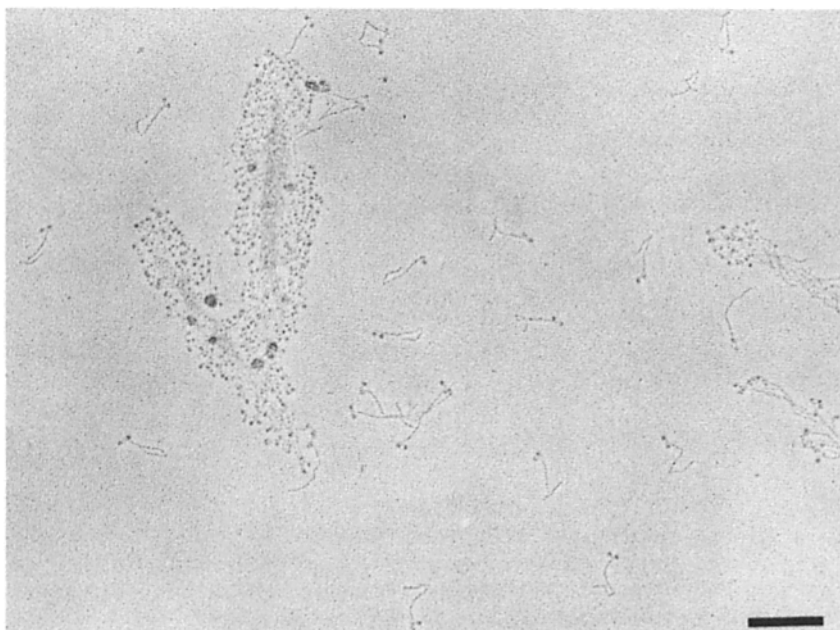
Figure 8. Bent *Dictyostelium* myosin monomers are excluded from filaments formed in 10 mM Tris, 1 mM DTT, 1 mM EDTA, 2 mM MgCl$_2$, pH 7.5, 60 mM KCl, and 2 mM MgCl$_2$ pH 7.5. Parallel dimers are rare under these conditions. Myosin was allowed to assemble for 30 min at 22°C in the above buffer (10 mM Tris, 1 mM DTT, 1 mM EDTA, 60 KCl, 2 mM MgCl$_2$, pH 7.5). The filament size was heterogeneous. Thick filaments formed that lack a clear central bare zone but seem to be bipolar. We also detected smaller antiparallel loose filaments like those on the right side of the image. Bar, 0.2 μm.
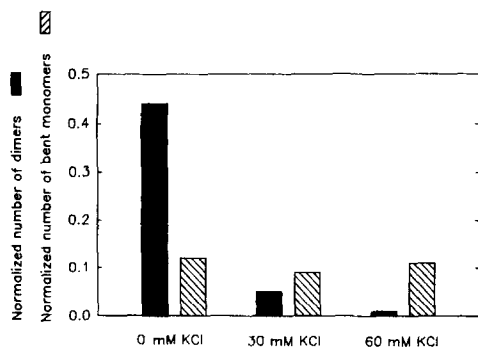
Figure 9. Bent monomers are excluded from myosin filaments formed in buffers containing 30 and 60 mM KCl. Monomers and dimers were counted and compared to ∼500 molecules of skeletal HMM molecules added as a standard. Conditions: 10 mM Tris, 1 mM DTT, 1 mM EDTA, pH 7.5 with 0 KCl, 30 or 60 mM KCl, purified *Dictyostelium* myosin without additional in vitro phosphorylation.

De Lozanne, 1988). Our visualization of myosin molecules staggered by 140 Å in a parallel dimer realizes the predictions based on theoretical calculations.

In low salt the region 750–1,250 Å from the head–tail junction (Fig. 4, segments *AD*) is the most common intermolecular contact in parallel dimers. The central 900–1,100-Å segment has the highest probability of being in contact regardless of the position of the molecule in the dimer. We believe that the procedures used to prepare the samples for EM do not affect the tail conformation or the degree of association among dimers. The stress of drying and the interaction with the mica surface do not seem to affect α-helical coiled coils (Flicker et al., 1982). Our measurements of contact between molecules in dimers were done using images of proteins under very low ionic strength conditions where large changes in ionic concentration during drying are unlikely. Furthermore, the following studies using complementary approaches support our conclusions and in fact make a more narrow distinction of a region important for assembly. An "HMM-like" fragment of *Dictyostelium* myosin does not assemble (Peltz et al., 1981). About 50% of the *AD* region mentioned above is included in two *Dictyostelium* myosin tail fragments that have been expressed in *Escherichia coli* (De Lozanne et al., 1987; O'Halloran et al., manuscript submitted for publication). The fragments have molecular masses of 58 kD (740 Å long) and 34 kD (425 Å long) and extend from ∼980 to 1,700 Å and 980 to 1,400 Å from the head–tail junction, respectively. These fragments are insoluble in low salt and assemble into paracrystals with a periodicity of 140 Å. In a previous study using differential solubility of proteolytic fragments and mapping with monoclonal antibodies, the segment important for assembly in *Dictyostelium* myosin was located in the region 930–1,500 Å from the head–tail junction (Pagh et al., 1984). All of these results using tail fragments suggest that the entire *A–D* region is not required for assembly; the carboxy-terminal half (980–1,250 Å) may be sufficient.

The highly regular repeating pattern of charged residues throughout the *Dictyostelium* myosin tail is interrupted only at two sites (Warrick et al., 1986) located 1,060 and 1,220 Å from the head–tail junction, assuming a helical rise of 1.485 Å/residue (McLachlan and Karn, 1982). Since these
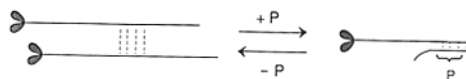
Figure 10. Schematic drawing of the possible relationships between the parallel dimer and the bent monomer. In this scheme, intramolecular contacts in the bent monomer (*broken lines*) compensate for the intermolecular contacts that stabilize the dimer. Phosphorylation of the tail provides the energy change that favors the bent monomeric form.

sites fall within the 980–1,250-A region discussed above, they may play a role in formation of the staggered dimer. The myosin tail may also be more flexible at these sites to allow bending. Flexible regions have been detected at ∼1,020 Å (Claviez et al., 1982; Kuczmarski et al., 1987) and, as we show here, at 1,200 Å from the head–tail junction in this study.

Parallel dimers can associate into antiparallel tetramers. The last 450 Å of the carboxyl-terminal part of the tail appears to form the antiparallel contacts. This region is distinct from that involved in the formation of parallel dimers. A study using monoclonal antibodies is consistent with the importance of the end of the tail in antiparallel interactions. Only small parallel aggregates form in the presence of a monoclonal antibody against *Dictyostelium* myosin that binds at the carboxy terminus of the tail (Pagh and Gerisch, 1986).

The portion of the myosin tail that contains the assembly and phosphorylation sites is clearly essential for the proper function of myosin in the cell. Cells that express a myosin lacking this portion of the tail (De Lozanne and Spudich, 1987) have properties similar to myosin null mutants (Manstein et al., 1989). Heavy chain phosphorylation is a physiological process that may regulate myosin assembly and play a role in filament relocation and subunit exchange.

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