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Structure-Function Studies on *Acanthamoeba* Myosins IA, IB, and II

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Myosins IA and IB are globular proteins with only a single, short (for myosins) heavy chain (140,000 and 125,000 daltons for IA and IB, respectively) and are unable to form bipolar filaments. The amino acid sequence of IB heavy chain shows 55% similarity to muscle myosins in the N-terminal 670 residues, which contain the active sites, and a unique 500-residue C-terminus highly enriched in proline, glycine, and alanine. The C-terminal region contains a second actin-binding site which allows myosins IA and IB to cross-link actin filaments and support contractile activity. Myosins IA and IB are regulated solely by phosphorylation of one serine on the heavy chain positioned between the catalytic site and the actin-binding site that activates ATPase.

Myosin II is a more conventional myosin in composition (two heavy chains and two pairs of light chains), heavy chain sequence (globular head 45% identical to muscle myosins and a coiled-coil helical tail), and structure (bipolar filaments). The tail of myosin II is much shorter than that of other conventional myosins, and it contains a 25 amino acid sequence in which helical structure is predicted to be weak or absent. The position of this sequence corresponds to the position of a bend in the monomer. Myosin II heavy chains also have a 29-residue nonhelical tailpiece which contains three regulatory, phosphorylatable serines. Phosphorylation at the tip of the tail regulates ATPase activity in the globular head apparently through an effect on filament structure.

Key words: actomyosin, cell motility, phosphorylation, filaments

In 1973 [1,2], we reported that *Acanthamoeba castellanii* contains a protein with myosin-like enzymatic properties but with physical properties unlike those of any previously described myosin; in 1979 [3], a second form of this atypical myosin was described. Both myosins contain only one heavy chain, of relatively low molecular weight, and one light chain. For this reason, and because both remain monomeric under conditions in which all other myosins form filaments, they were named myosin IA and IB; IA has a heavy chain of Mr 140,000 and a light chain of Mr 17,000, while IB has a heavy chain of Mr 125,000 and a light chain of Mr 27,000 (all by SDS-gel

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electrophoresis). Myosins IA and IB bind tightly to F-actin [1–3] and express actin-activated Mg^{2+}-ATPase activity when one serine residue on the heavy chain is phosphorylated by a kinase that has been purified to homogeneity [4,5]. While these studies were in progress, a more typical myosin was also found to be present in *Acanthamoeba* [6,7]. Myosin II contains a pair of heavy chains of Mr 175,000 and two pairs of light chains of Mr 17,500 and 17,000 (all by SDS-gel electrophoresis). Myosin II forms bipolar filaments [8,9] and its actin-activated Mg^{2+}-ATPase activity is inhibited when three serine residues on each heavy chain are phosphorylated by a partially purified kinase [10,11]. These data are summarized in Table I. In this article, we review the current status of the work from our laboratory on these three myosin isoenzymes.

We recognized very early the need to prove that myosins IA and IB are not two forms of the same enzyme, that neither is derived from myosin II, and that none of the three myosins is derived from a still more typical myosin (with larger heavy chains than those of myosin II). The initial conclusion, from peptide mapping [12] and immunochemical specificity [13], that these enzymes were, indeed, products of three different genes was confirmed unequivocally by the identification of a mRNA for each heavy chain [14]. These latter experiments also established that the molecular weights of the isolated proteins are those of the native enzymes. That myosins I and II are unrelated was, of course, inherent in the fact that they are phosphorylated by different kinases [4,5,15–17] (although the same phosphatase [18,19] can dephosphorylate both enzymes) and with opposite effects on their actin-activated ATPase activities.

AMINO ACID SEQUENCE OF MYOSIN IB HEAVY CHAIN

The next question to be addressed was whether myosins IA and IB are truly myosins. This question became even more interesting when we found that *Dictyostelium discoideum* contains a very similar monomeric myosin I [20] and when others [21] recognized that the 110,000-dalton protein from intestinal brush border microvilli has myosin-like properties. The question was answered by isolating and sequencing a genomic clone of myosin IB heavy chain [22,23]. The gene spans 6 kilobases (kb) and contains 23 exons that code for polypeptide of 1,168 amino acids (Mr 127,800). When compared to the sequence of rat embryonic skeletal and nematode muscle

TABLE I. Summary of the Properties of the *Acanthamoeba* Myosins

Molecular weight[a]	159,000	150,000	450,000
Subunits[b]	140,000 (1) 17,000 (1)	125,000 (1) 27,000 (1)	175,000 (2) 17,500 (2) 17,000 (2)
Filaments	No	No	Yes
Phosphorylation sites[c]	1/heavy chain	1/heavy chain	3/heavy chain
Actin-activated Mg-ATPase, s^{-1}	11[d]	17[d]	3.7[e]

[a]Sedimentation equilibrium values.
[b]Sodium dodecyl sulfate-gel electrophoresis values.
[c]All phosphorylation sites are serine residues.
[d]V_{max} when heavy chain is phosphorylated.
[e]V_{max} when heavy chains are unphosphorylated.

myosin heavy chains [24,25], the first 80 amino-terminal amino acids (a highly variable region of muscle myosins) are absent, but the next 670 residues show 55% similarity, including exact matches and conservative changes. This sequence similarity is illustrated graphically in Figure 1. Particularly strong similarities exist in the region thought to be involved in nucleotide binding and in the "active thiol" region. Furthermore, the positions of 9 of the 15 introns in this segment of the gene are identical with the positions of introns in the rat embryonic skeletal muscle myosin heavy chain gene [22–24]. There are, however, some potentially interesting sequence differences in this domain of the heavy chain; for example, the two cysteines that give the active thiol region its name are missing from myosin IB, and there is little similarity betweeen the amoeba and muscle genes in the region that is thought to represent a flexible swivel in muscle myosin heavy chain connecting the head and tail.

In contrast to the strong similarity between the amino-terminal 670 residues of the myosin IB heavy chain and the globular head sequences of muscle myosins, the carboxyl-terminal 500 residues of the myosin IB heavy chain do not show any significant similarity to any portion of muscle myosin sequences. Furthermore, this unique carboxyl-terminal domain lacks completely the heptad repeat of hydrophobic residues characteristic of the rod sequence of conventional myosins (and all proteins which form α-helical coiled coils [26]). This unique carboxyl-terminal domain also contains two regions which are rich in glycine, proline, and alanine. A 56-residue subregion contains 40% glycine, 23% proline, and 9% alanine and the carboxyl-terminal 135 residues contain 36% glycine, 30% proline, and 16% alanine. All indications from the sequence are that this carboxyl-terminal, 500-residue domain is incapable of forming an α-helical coiled coil structure typical of conventional myosins. This is in agreement with the monomeric nature of myosin IB and its inability to self-assemble into filaments.

Therefore, the sequence data definitively establish that myosin IB is a "myosin" in the globular head domain that contains the catalytic activity but has a unique, non-

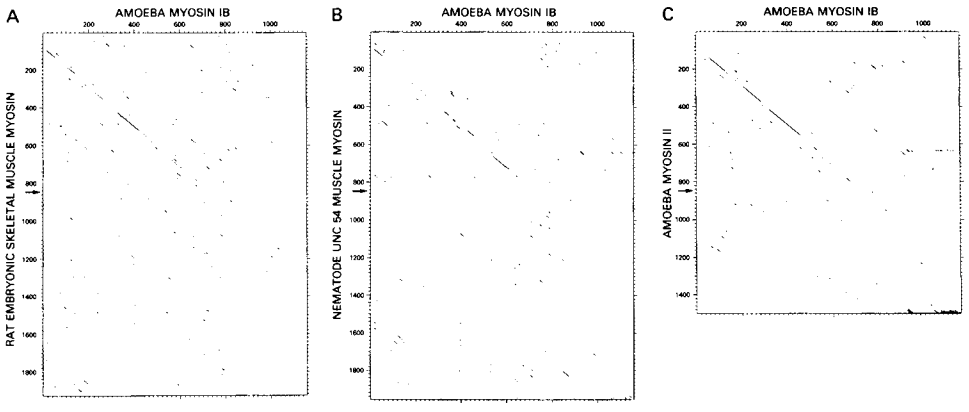

Fig. 1. Dot matrix comparison of the amino acid sequence of *Acanthamoeba* myosin IB heavy chain to the sequences of the heavy chains of **A)** rat embryonic skeletal muscle myosin, **B)** nematode muscle myosin, and **C)** *Acanthamoeba* myosin II heavy chains. Identical residues lie on the diagonal. The arrow on the Y-axis of each figure indicates the position of the proline that separates the head from the tail. (Data are from Jung et al [23].)

myosin sequence in the carboxyl-terminal segment, rather than the rod-like tail structure that confers upon muscle myosins the ability to associate into bipolar filaments. Comparable sequence data are not yet available for myosin IA heavy chain but partial data at the protein level (to be discussed later) are fully consistent with this picture.

MYOSINS IA AND IB CONTAIN TWO ACTIN-BINDING SITES

Both myosin IA and IB can support analogues of contractile activity in vitro. Latex beads coated with myosin I translocate along actin cables [27] and actomyosin I complexes undergo superprecipitation [28]. Both activities are ATP dependent and occur only with phosphorylated myosin IA and IB (the state that expresses actin-activated Mg^{2+}-ATPase activity).

The first insight into the mechanism by which monomeric myosin I can support superprecipitation, a property previously believed to require the presence of bipolar filaments, came from studies of the actin dependence of myosin I Mg^{2+}-ATPase activity. Triphasic behavior was found [2,29,30]: activation at low concentrations of F-actin, inhibition at intermediate actin concentrations, and re-activation at higher actin concentrations (Fig. 2). Binding assays showed that all of the myosin was bound, and remained bound, to F-actin throughout the entire range of actin concentrations [30]. Double reciprocal plots of these data were linear over both regions of activation with the same values for Vmax but, of course, very different values for the actin concentration required for half-maximal activity (K_{ATPase}). These unusual kinetics could be explained [30,31] by assuming that myosin I contains two actin-binding sites, one associated with the catalytic site which is responsible, as for other myosins, for actin-activated Mg^{2+}-ATPase, and one that serves only to cross-link actin filaments with no effect on ATPase activity. At high concentrations of actin, the myosin:actin ratio will be very low and the myosin molecules wil be widely separated along the actin filaments that they cross-link. Under these conditions, the actin-

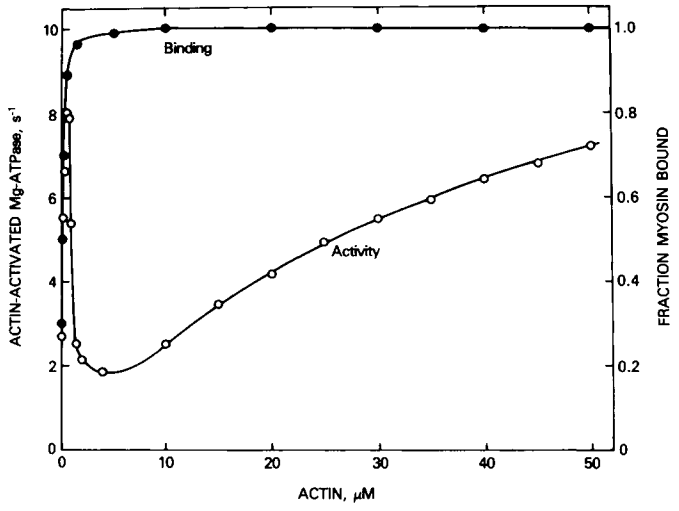

Fig. 2. Actin concentration dependence of the binding of *Acanthamoeba* myosin I to F-actin and its actin-activated Mg^{2+}-ATPase activity. (Data are from Albanesi et al [30].)

activation curve will be a direct reflection of the K_{ATPase} of the site associated with activation. At very low actin concentrations, the myosin:actin ratio will be very high, and this will lead to cooperative binding of relatively closely packed myosin molecules along the cross-linked filaments. Therefore, under these conditions, activation will occur at actin concentrations very much lower than the nominal K_{ATPase}. At intermediate values, the actin concentration will be too far below the K_{ATPase} to activate directly and too high to allow cooperative binding of myosin. This hypothesis is supported by the finding that shortening the actin filaments inhibits the cooperativity, as measured by the actin-dependence of myosin I Mg^{2+}-ATPase [32]. Furthermore, cross-linking the actin filaments by other proteins [33] enhances cooperativity. Physical evidence for cross-linking was obtained by the increase in low-shear viscosity [28] and by electron microscopic visualization of filaments cross-linked by molecules of the dimensions of myosin I [34].

The cooperative, cross-linking model predicts that the heavy chain should contain two, possibly separable, actin-binding sites. We do not need to consider the light chain in this regard because we have shown that the heavy chain alone has the same enzymatic properties as the native molecule [35]. Limited chymotryptic-cleavage of myosin IA produces a 112-kilodalton (kDa) amino-terminal peptide and a carboxyl-terminal 27-kDa [34]. The 112-kDa peptide contains the catalytic site [34,36,37] and the regulatory phosphorylation site [34,37] and, when phosphorylated, has full actin-activated ATPase activity [34] but with normal Michaelis-Menten kinetics. This peptide binds to F-actin in an ATP-sensitive manner (K_D = 6.7 nM and 0.5 μM, in the absence and presence of ATP) but cannot cross-link actin [34]. The 27-kDa peptide binds to actin with equal affinity in the presence and absence of ATP (K_D = 0.12–0.34 μM), also does not cross-link actin, but has no enzymatic activity [34]. Consistent with the derived amino acid sequence of myosin IB, the carboxyl-terminal 27-kDa peptide of myosin IA [34] has a very high concentration of glycine (34%), proline (21%), and alanine (12%). We conclude that the heavy chain of myosin I contains two actin-binding sites—one, as for all myosins, is ATP-sensitive and is located in the globular head near the catalytic site which it activates; the other is located in the non-helical carboxyl-terminal 25% of the heavy chain that is unique, among the myosins thus far characterized, to the myosin I of *Acanthamoeba* and has no direct effect on ATPase activity.

MODEL FOR CONTRACTILE MECHANISM INVOLVING MYOSINS IA AND IB

With this information, we could propose a reasonable model for the mechanism by which monomeric, non-filamentous myosin IA and IB might support contractile activity in situ [34]. The myosin monomer would cross-link actin-filaments through an ATP-independent binding site and an ATP-sensitive binding site associated with catalysis. The ATP-insensitive site would act as a hinge or fulcrum about which the myosin molecule would rotate as the ATP-sensitive site cyclically associated and dissociated from the actin coordinately with the ATPase cycle. Formally, this model is more analogous to the microtubule-dynein cycle [38] than to the muscle actomyosin cycle [39]. It is also possible that myosin I also, or only, functions analogously to microtubule-dependent translocators [40,41] and is responsible for the movement of vesicles along actin cables [27,42]. The apparent preferential localization of myosin I

in the cell periphery [43] and the isolation of a cytoskeleton fraction enriched for myosin I [44] are compatible with both possible functions.

FUNCTIONAL DOMAINS OF MYOSINS IA AND IB

One of our current goals is to establish the structural basis for actin activation of the myosin I Mg^{2+}-ATPase activity and the mechanism by which phosphorylation of the heavy chain regulates the actin-activated ATPase activity. It is useful to remember, in this regard, that the three *Acanthamoeba* isoenzymes are the only myosins thus far described in which regulatory light chain phosphorylation does not occur. We have already mentioned that chymotrypsin-cleavage of myosin IA produces a 112-kDa amino-terminal peptide with full actin-activated ATPase activity dependent on its being phosphorylated.

More extensive proteolysis by trypsin and *Staphylococcus aureus* protease (V8) has led to additional insights into structure-function relationships within the myosin IA heavy chain. Some of the major sites of proteolytic cleavage are indicated schematically in Figure 3. The ATP-binding site resides in the amino-terminal 27 kDa and the regulatory phosphorylation site resides in the segment between 38 and 58 kDa from the amino-terminus [45]. An actin-binding site, analogous to that found in muscle myosin subfragment 1, has been tentatively identified by zero-length cross-linking as being carboxyl-terminal to the phosphorylation site. Consistent with this localization, a site 64 kDa from the amino-terminus is protected from trypsin digestion when the myosin is bound to actin. The second actin-binding site in the carboxyl-terminal 27-kDa fragment has been described above.

Under controlled conditions, trypsin digestion produces an amino-terminal 38-kDa peptide and central 74-kDa peptide which remain associated with each other and with the light chain under non-denaturing conditions. This complex is chemically similar to the chymotryptic 112-kDa fragment/light chain complex but for the cleavage at the 38/74 junction. While the 38/74 complex retains full (K^+,EDTA)ATPase, its Mg^{2+}-ATPase is not fully activatable by actin. The 38/74 complex retains the low level of actin-activated Mg^{2+}-ATPase characteristic of unphosphorylated myosin IA or its 112-kDa subfragment, but this activity is not increased by phosphorylation. The 38/74 complex also binds actin in an ATP-sensitive manner with affinities similar to

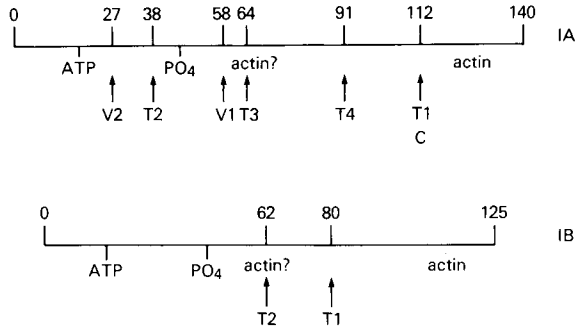

Fig. 3. Schematic representations of the proteolytic cleavage sites in the *Acanthamoeba* myosin IA and IB heavy chains. C, chymotrypsin; T, trypsin; V, *Staphylococcus aureus* protease V8. The numbers following the symbols indicate the order of susceptibilities of the different sites.

those of the unphosphorylated 112-kDa fragment. Cleavage at the 38/74 junction separates the nucleotide-binding site from the phosphorylation and actin-binding sites, but it is clear that the nucleotide- and actin-binding sites are still able to influence each other. It is rather that the phosphorylation site has been uncoupled from the process by which binding to actin activates the Mg^{2+}-ATPase of myosin I. The region about the 38/74 junction obviously plays an essential role in the full actin-activation of myosin IA. Consistent with this conclusion, the proteolytic susceptibility of the 38/74 junction is altered by heavy chain phosphorylation, but only when the myosin IA is bound to F-actin.

Trypsin-cleavage of myosin IB heavy chain proceeds differently than cleavage of myosin IA heavy chain and provides additional information (Fig. 3). Digestion in the presence of F-actin produces an 80-kDa peptide with full actin-activated ATPase activity that is still regulated by phosphorylation [46]. This peptide no longer cross-links F-actin. When the 80-kDa peptide is dissociated from the actin by addition of MgATP, the trypsin digestion proceeds further producing an N-terminal 62-kDa peptide (which suggests that actin binds in this region) which retains an active catalytic site and the phosphorylation site and still binds to F-actin, but which has no actin-activated ATPase activity. Thus, at least a portion of the 18-kDa segment that lies between 62 kDa and 80 kDa of the amino-terminus is essential for actin-activated Mg^{2+}-ATPase activity of myosin IB. As mentioned earlier, this segment is homologous to the active thiol region of muscle myosins, although the two reactive cysteines are missing.

AMINO ACID SEQUENCE OF MYOSIN II HEAVY CHAIN

The complete myosin II heavy chain gene spans 6 kb and contains only three small introns at positions corresponding to introns in characterized vertebrate and invertebrate muscle myosin heavy chain genes [47,48]. The coding sequence for 1,509 amino acids (Mr 170,966) shows about 45% identity with muscle myosins in the N-terminal 847 residues corresponding to the globular head with particularly strong similarities in the regions generally thought to contain the catalytic, actin-binding, and light-chain binding sites. There is little similarity between the myosin II and muscle myosin sequences in regions of low sequence similarity between the muscle myosin heavy chains. In contrast to myosin I, the similarity between myosin II and muscle myosin heavy chains continues into the tail. Direct sequence identity does not occur in this region, but there is strong chemical similarity. In all cases there is a repeating heptad, with hydrophobic amino acids usually occurring at intervals of 3 and 4, and 28-amino acid residue zones with the same characteristic patterns of negatively and positively charged amino acids as in muscle myosins. This sequence predicts that the two myosin II heavy chains will form a coiled-coil helix, as observed [9,42,50], through which myosin II molecules will associate into bipolar filaments, also as observed [49–51].

There are, however, at least three major differences between the rod sequences of myosin II and the muscle myosins [48]: a) Myosin II tail contains only 25 full and partial 28-residue zones (in contrast to 41 for nematode myosin); this accounts fully for the difference in the molecular weights of the two heavy chains and for the differences in lengths of their tails (86–90 nm [48,49] vs 160 nm [26]). b) Myosin II tail contains a 25-amino acid region about 60% of the way between the head-tail

junction and the carboxyl-terminus which contains a proline residue and disruptions in the hydrophobic heptad repeat; the helical coiled-coil structure in this region is likely to be weak or absent. c) The carboxyl-terminal 29 amino acids of myosin II are predicted to be entirely in a non-helical structure. Interestingly, this non-helical, carboxyl-terminal tailpiece contains the three previously identified [16,17] regulatory phosphorylation sites: serine residues at positions 11, 16, and 21 from the carboxyl-terminus [52]. And, from the similarity of surrounding sequences [47], the serine at position 5 from the carboxyl-terminus provides a potential fourth phosphorylation site.

MECHANISM OF MYOSIN II REGULATION BY PHOSPHORYLATION

The major question we have addressed with respect to myosin II is how phosphorylation of 3 (or 4) serines at the very tip of the tail can inhibit the actin-activated Mg^{2+}-ATPase activity that resides in the globular head [53] (see Fig. 4). Our first relevant observations were that myosin II is filamentous under all conditions in which actin-activated Mg^{2+}-ATPase activity occurs [8,50,54] and that, although both phosphorylated and non-phosphorylated mysoin II are filamentous (and have Ca^{2+}-ATPase activity and bind to F-actin), filaments of phosphorylated myosin II are always smaller under identical conditions [54]. These observations suggest that regulation of actin-activated Mg^{2+}-ATPase activity of myosin II might occur through a change in filament conformation [50,54,55]. Strong support for this hypothesis is provided by the observation that copolymers of phosphorylated and non-phosphorylated myosin II have much less actin-activated Mg^{2+}-ATPase activity than a mixture of homopolymers of equivalent overall composition; ie, phosphorylated myosin II

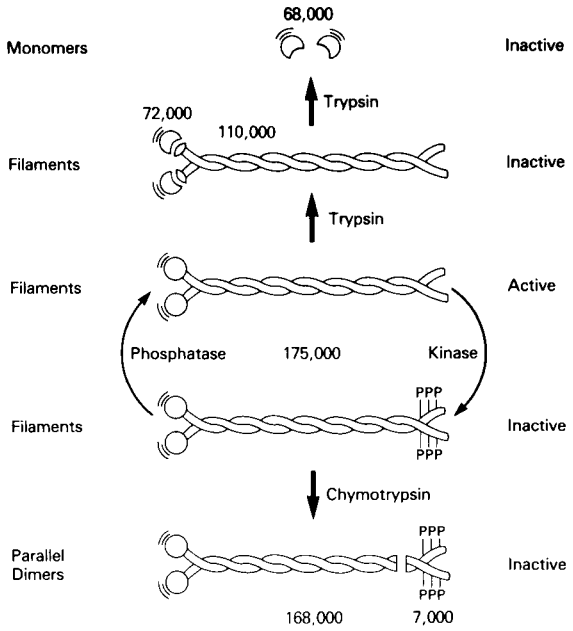

Fig. 4. Schematic summary of the structure-function studies on *Acanthamoeba* myosin II that are discussed in the text.

inhibits non-phosphorylated myosin II when they are present in the same filament but not when they are in separate filaments [54].

We next made use of the fact that limited chymotrypsin digestion of myosin II selectively removes the carboxyl-terminal 66 amino acids [53] with no other effects on the heavy chains or on either pair of light chains [49] (Fig. 4). The chymotrypsin-cleaved myosin II has full Ca^{2+}-ATPase activity and binds to F-actin apparently normally but has no actin-activated Mg^{2+}-ATPase activity [49]. Analytical ultracentrifugation and electron microscopy showed that the chymotrypsin-cleaved molecule forms only parallel dimers under the enzyme assay conditions in which native myosin II forms filaments [49]. Thus, this short segment at the end of the heavy chain, which contains about 40 amino acids contributing to the helical coiled-coil structure in addition to the nonhelical carboxyl-terminal tailpiece, is clearly very important both for formation of bipolar filaments and for actin-activated Mg^{2+}-ATPase activity. This suggests, but does not prove, that filaments are required for the actin-activated Mg^{2+}-ATPase activity of myosin II to be expressed. This hypothesis is further strengthened by observations by Kiehart and Pollard [56] that monoclonal antibodies to the carboxyl-terminal region of the tail depolymerize myosin II filaments and inactivate their actin-activated Mg^{2+}-ATPase activity and our demonstration [57] that polyclonal antibodies raised against a synthetic peptide with the sequence of the helical region immediately preceding the non-helical tailpiece have similar properties.

Therefore, it seems reasonable to conclude that the state of phosphorylation of the carboxyl-terminal non-helical tailpiece regulates the actin-activated Mg^{2+}-ATPase activity of myosin II by altering the conformation of the filament in such a way that the myosin heads, although still able to bind to F-actin, cannot proceed through a normal catalytic cycle.

But filaments are not essential for actin-activated Mg^{2+}-ATPase activity under all experimental conditions. Trypsin cleaves myosin II heavy chain in the head region (Fig. 4) producing a carboxyl-terminal 110-kDa peptide and an amino-terminal 72-kDa peptide [50] that can be further degraded to an amino-terminal 68-kDa peptide with associated, native light chains [58]. Parenthetically, the retention of both light chains by the 68-kDa heavy chain fragment is interesting because this peptide is missing the segment thought to be involved in light-chain binding by muscle myosin heavy chains [59]. The 68-kDa head fragment, like the much larger chymotrypsin-cleaved myosin II, retains full Ca^{2+}-ATPase activity and binds apparently normally to F-actin but has no actin-activated Mg^{2+}-ATPase activity. However, when covalently cross-linked to F-actin, full actin-activated Mg^{2+}-ATPase activity is recovered [58]. Therefore, bipolar filaments are not absolutely necessary for myosin II to express functional ATPase activity, but they are likely to be required for native myosin II under conditions in the cell.

STRUCTURE OF MYOSIN II MONOMERS AND DIMERS

Recently, we have directed more attention to determining the structure of myosin II filaments with the goal of seeking structural differences between the phosphorylated and unphosphorylated enzymes that might explain their functional differences. To learn something about the solution structure of the monomer, we began with studies of the chymotrypsin-cleaved myosin II, which would not be complicated by the formation of filaments, and we utilized electric birefringence measurements [60]. In

this method, molecules with a permanent or electrically inducible dipole are oriented in an electric field. For asymmetric molecules such as myosin, this creates an anisotropy that can be quantified by optical birefringence measurements. When the induced birefringence reaches a stable plateau, the current is turned off and the time course of the decay in birefringence is measured. From the shape of the curve, the number of components contributing to the decay can be determined, and from their relaxation times, the rotational diffusion constants and, hence, the geometry of each component can be calculated.

Over a range of salt concentrations, two species of chymotrypsin myosin II are detected [60]: one with a relaxation time of 8.2 μsec and one with a relaxation time of 25 μsec, corrected to water at 25°C. In 100 mM KCl, the former accounts for 88% of the total and, in the absence of KCl, the solution comprises 70% of the latter species. The logical conclusion is that these represent monomer and dimer, respectively. Theoretical calculations show that a monomer with a straight, rigid rod would have a relaxation time of 9.9 μsec, leading to the necessary conclusion that the 8.2 μsec species must be a bent monomer. However, these data do not, in and of themselves, provide unique values for either the position of the bend or the angle of the bend, which are interrelated variables. But electron microscopic images of negatively stained preparations consistently reveal a bend 26 nm from the end of the 76-nm long tail of chymotrypsin-cleaved myosin II [49] and 36 nm from the end of the 86-nm long tail of native myosin II [48]. The position of this bend corresponds precisely to the calculated position of the weakened helical structure in the coiled-coil rod predicted from the derived amino acid sequence [48]. On the reasonable assumption that this is the position of the bend in all molecules in solution, an average bend angle of 110° for chymotrypsin-cleaved myosin II monomers (Fig. 5) is calculated from the electric birefringence data. It should be noted that if this bend position is structurally analogous to the hinge region between the light meromyosin and subfragment-2 segments of muscle myosin [6], myosin II has a much shorter light meromyosin segment and a slightly longer subfragment-2 segment than muscle myosin. Similar analysis of the relaxation time of the dimer leads to the conclusion that it is a parallel dimer with straight tails and a displacement of 28 nm between the two monomers [61] (Fig. 5). Electron microscopic images are consistent with this interpretation [49]. In contrast to these results, dimers of rabbit skeletal muscle myosin are staggered by 43 nm [62].

MODEL FOR ASSEMBLY AND STRUCTURE OF MYOSIN II FILAMENTS

We can use these structural data to propose [63] a reasonable model for the assembly of myosin II filaments (Fig. 5) and, much more speculatively, how the filaments might be regulated by phosphorylation. The monomer is illustrated as two globular heads (with associated light chains) and a 86-nm coiled-coil helical tail with a potential hinge region 36 nm from the carboxyl-terminus. Three domains can be distinguished within the coiled-coil rod: a 50-nm region amino-terminal to the bend, a 26-nm region between the bend and the chymotrypsin-sensitive site, and a carboxyl-terminal 10-nm region that is removed by chymotrypsin cleavage. The last 29 residues of the tail compose a non-helical tailpiece with three (or four) regulatory, phosphorylatable serines.

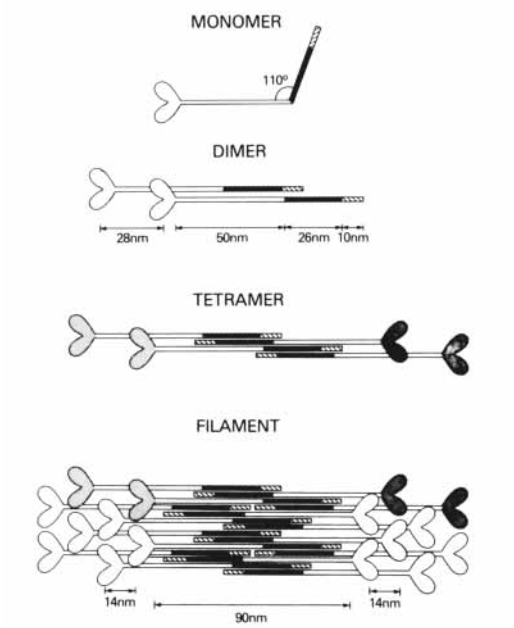

Fig. 5. Proposed model for the assembly of *Acanthamoeba* myosin II into bipolar filaments. The molecule is represented as two globular heads and a tail divided into three segments: 1) the coiled-coil helix from the heads to the bend position, 2) the coiled-coil helix between the bend position and the segment removed by chymotrypsin, and 3) the segment removed by chymotrypsin which includes the remaining coiled-coil and the non-helical tailpiece. See text for details.

From the birefringence data, monomers associate into a parallel dimer, with a stagger of 28 nm (Fig. 5). We propose that the parallel dimers assemble into an antiparallel tetramer; for this reaction, interactions involving the 10-nm, terminal, helical 41 amino acids are essential. We propose that then, either by self-association of tetramers* or stepwise addition of dimers, a filament of 16 myosin monomers is formed with the dimensions calculated by Pollard [51] from electron microscopic images of negatively stained filaments of myosin II. In the model, the intercalcated heads are separated by 14 nm, compared to 15 nm in Pollard's measurements, and the model has the twofold rotational symmetry seen by electron microscopy and consistent with the 6–7-nm diameter of the central bare zone [51]. With this twofold rotational symmetry (in contrast to the threefold rotational symmetry of muscle myosin [61]), the filaments are necessarily self-limiting in length, as observed experimentally [8,9,49–51]. The structural basis of this length constraint, which does not occur in muscle myosin [61], may be related to the source of the difference in the stagger between the monomers of the amoeba and muscle dimers. In the proposed filament structure, every myosin II molecule is involved in antiparallel interactions, in contrast to muscle myosin filaments in which a central antiparallel domain is

*Parenthetically, it is of some interest that bipolar minifilaments of skeletal muscle myosin (which contain 16 or 18 monomers) seem to be in equilibrium with antiparallel octamers and tetramers (which may, in turn, be formed from parallel dimers [64]). It is possible that filaments of myosin II are formed by association of tetramers to form octamers and then of octomers with each other.

flanked on both sides by extensive domains involving only parallel interactions between myosin molecules. This may explain why chymotrypsin-cleaved myosin II, which lacks the 10-nm, carboxyl-terminal segment of the coiled-coil rod that is apparently essential for antiparallel interactions, is unable to co-polymerize with native myosin II.

In the assembled filament, there are multiple potential interactions between the regulatory phosphorylation sites of one monomer and other regions of the heavy chains of other monomers. The precise interactions will depend on the length of the bare zone in the middle of the bipolar filament. The model is consistent with Pollard's measurements, allowing a minimum bare zone of 90 nm and maximum bare zone of about 100 nm. As drawn, the phosphorylation sites of one monomer in the parallel dimer lie about 8 nm on the N-terminal side of the potential bend position of the other monomer. Phosphorylation sites of one parallel dimer lie within about 4 nm on the N-terminal side of the potential bend position of one monomer of the other dimer in the antiparallel tetramer. In a filament with a 90-nm bare zone, additional potential interactions are created between phosphorylation sites and the 50-nm N-terminal helical portion of the rod and between the phosphorylation-site regions of oppositely oriented molecules.

Because the non-helical tailpiece contains five arginine and two terminal glutamate residues, phosphorylation of the three or four serines in this tailpiece can lead to a change in charge from +2 to about −2. This extensive alteration of charge could be accompanied by a change in conformation of the filaments around the bend (hinge) position such that the globular heads are no longer able to interact with F-actin in a catalytically functional way. It is interesting that the studies in vitro of the actin-activated ATPase of myosin II as a function of phosphorylation [16] show that the transition from fully active to fully inactive enzyme occurs when the phosphate content increases from 1.5 to 2.5 per heavy chain, which, assuming one negative charge per phosphate, is the region over which the net charge in this region changes from +0.5 to −0.5 (Fig. 6). To establish the validity of any of these possibilities will require extensive studies of the structures of phosphorylated and non-phosphorylated

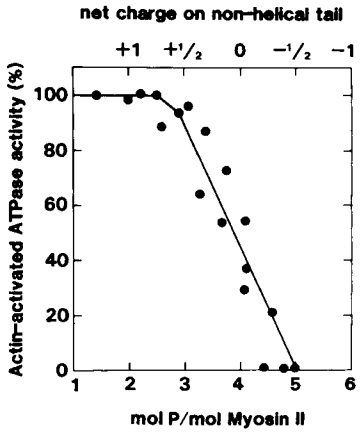

Fig. 6. Relationship between the actin-activated Mg^{2+}-ATPase activity of *Acanthamoeba* myosin II, the mol of P/mol of myosin, and the calculated net charge on the non-helical tailpiece. (Data are recalculated from Cote et al [16].)

myosin II monomers, dimers, and especially filaments with particular emphasis on the orientation of the globular heads as a function of phosphorylation state.

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