A MODEL FOR LENGTH-REGULATION IN THICK FILAMENTS OF VERTEBRATE SKELETAL MYOSIN

JULIEN S. DAVIS
Department of Biology, The Johns Hopkins University, 34th and Charles Streets, Baltimore, Maryland 21218

ABSTRACT A mechanism for length regulation in the parallel-packed section of the thick filament is proposed. It is based on experiments done on synthetic, mini- and native filaments, and its primary purpose is to explain the physical basis of the kinetic mechanism for the assembly of synthetic thick filaments from myosin alone. Kinetically, length is regulated by a dissociation rate constant that increases exponentially as the filament grows bi-directionally from its center. Growth ceases at the point of equilibrium between invariant on and length-dependent off rates. The three subfilament structure of the parallel-packed region of the thick filament is fundamental to the proposed scheme. The intra-subfilament bonding is strong and predominantly ionic in character, whereas the inter-subfilament bonding is relatively weak. These strong and weak interactions participate directly in the strictly sequential mechanism of assembly of dimer subunit observed in the kinetics. A third domain, independent of the sequential mechanism, consists of opposing negative charges on the subfilament surface, juxtaposed at or close to the thick filament axis. The weak and repulsive domains are additively coupled to each other through the rigidity in the subfilaments. Length regulation occurs through the repulsive component rising in intensity more rapidly with length than the initially stronger positive interactions. Growth ceases at the point where the repulsive interactions weaken the attractive interactions to the extent that equilibrium is established between head-to-tail dimer subunit and its binding sites at the tips of the arms of thick filament. This myosin-mediated mechanism, which gives rise to a narrow length distribution, is considered to be fine-tuned by co-polymerizing proteins to give the precise length of the native filament.

INTRODUCTION

This paper describes the complex role that the rodlike tail of the myosin molecule plays in thick filament assembly. A study of this process is of value in understanding the complexities of supramolecular structure generation. The kinetics of synthetic thick filament assembly (1–4) has presented us with a well-characterized mechanism for the way in which a complex three-dimensional structure of self-limiting size is generated from one protein (5, 6). Length is regulated by a dissociation rate constant that increases exponentially as the parallel-packed arms grow bi-directionally from the thick filament center. Growth ceases at the point of equilibrium between a length-independent on rate and a length dependent off rate for the interaction of myosin dimer with the two tips of the thick filament (2–4). A mechanism of this type relies on emergent properties for its mode of operation, assembly being directed by the ever-changing intrinsic properties of the growing polymer. It belongs to the larger class of cumulative strain mechanisms (7–9). This paper is concerned with the physical basis of the mechanism.

A striking feature of the thick filament of vertebrate skeletal muscle, viewed at the resolution of an electron microscope, is its precise length. Various mechanisms have been proposed to explain how this precise 1.6-μm length is achieved. Co-polymerizing proteins unquestionably interact with the myosin-mediated mechanism described above, to generate the native filament structure from the narrow length distribution produced by the myosin molecule alone. The filament itself is bipolar, i.e., assembled bi-directionally from a common origin giving rise to twofold rotational symmetry about its center. This orientation of the subunits is required for its functional role in which the cycling cross-bridges have to pull the thin filaments of actin protein of a sarcomere toward each other to cause contraction. The myosin subunit is a highly asymmetric molecule in which two globular ATP-cleaving heads are attached to a long rod. It is the rod portion of the molecule (an α-helical coiled-coil) lying distal to the pair of heads that generates the backbone of the thick filament. The bipolar structure originates in the antiparallel packing of myosin at the center of the filament, which gives rise to the characteristic bare zone. The cross-bridges of skeletal striated muscle appear at either side of this bare zone as a three-start right-handed helical array (10) with an axial repeat of 43 nm. The mode of packing changes gradually, as the region of antiparallel overlap extends, to one with myosin molecules assembling in a uniform head-to-tail packed, parallel array. A comprehensive scheme for the specific way in which myosin packs in the shaft of the thick filament has yet to be devised.

Studies of the kinetics of assembly have helped to clarify this problem in recent years (1–6). Rather than attempt to unravel the complexities of native filament assembly, in which a number of co-polymerizing proteins are integrated at specific sites into the structure of the thick filament, the system was simplified by following the formation of synthetic thick filaments made from purified myosin. Since myosin is the predominant thick filament protein and is centrally located within the native structure, it seemed likely that these filaments would exhibit many of the characteristic features of the myosin-mediated assembly process. The main problem encountered in this approach was in selecting the correct synthetic thick filament system, of which a number of classes with distinct structural features exist. It turns out that in making this choice the main consideration was the preservation of the distribution of charged groups present on the protein in the physiological state. This realization came about after inspection of the structure of the different classes of thick filament. A class favored by researchers for in vitro studies were filaments generated at pH 7.0 by reducing the salt concentration by dialysis. In these studies the filaments were generally heterodisperse, with diameters greater than those of native filaments. On the positive side, these filaments did exhibit organized structure in the electron microscope and had mean length distributions similar to those observed in intact sarcomeres. The class of filaments generated at pH 8.0–8.5, at physiological salt concentrations, and at 5°C were by and large overlooked despite the presence of a homogeneous population of polymers that were in all major respects structurally similar to native filaments. These filaments were shorter than native filaments and exhibited a narrow length distribution (e.g., 0.65 S.D. $\mp$ 0.07 μm, n = 120 [4]) (11). The fact that a pH of 8.0–8.5 at 5°C was necessary for the formation of this class of filaments first focused attention on the fundamental importance of approximating the distribution of charged groups on the myosin molecule found at pH 7.2 and 39°C in the rabbit (4). The point here is not simply that the *pK* values of cationic acids change with temperature and have to be compensated for by a change in pH, but that a change in the charge pattern along the rod portion of the myosin molecule has a profound influence on the fidelity of synthetic and, by analogy, native thick filament assembly.

The specific role that charge plays in directing filament assembly and, in particular, thick filament length regulation has been emphasized in recent kinetic experiments in which the extent of the ionic interactions between adding subunit and growing filament changed with length (4). Here, two structural elements of the thick filament exhibit quite distinctive behavior in their interaction with parallel dimer (the assembly intermediate consisting of two myosin molecules associated with parallel array that adds to the filament ends). Growth of an experimentally defined segment of the central antiparallel region of the thick filament by the sequential addition of dimer is correlated with a linear increase both in the Gibbs energy of interaction and in the dominance of charge-charge interactions in assembly (4). After the change-over to parallel packing the Gibbs energy continues to increase in a linear fashion with size, but is now correlated with a linear decline in the extent of polar interactions. The kinetics and thermodynamics characteristic of parallel-packed myosin appear to extend across the boundary at $\mp$0.4 μm into the region perturbed by antiparallel overlap. Conservatively estimated, the graded change-over in the kinetics from that typical of parallel packing starts at the point where the 0.65-μm filament is 60% dissociated (4). The development of such cumulative, length-dependent properties necessitates interactions within the structure that transcend those that operate between neighboring subunits. That is, the size of the aggregate is regulated by the interaction of binding sites at the growing tips of the thick filament with the rest of the structure.

Since changes in the charge-charge interactions between macroscopic groupings of subunits within the thick filament appear to hold the key to the physical basis of the length regulation mechanism, we have examined the different structures that native and synthetic thick filaments could assume under conditions that maintained the physiological charge pattern as closely as possible (12–15). We made particular use of experiments in which electrostatic interactions were amplified simply by lowering the ionic concentration of the medium. The *pK* values of cationic acids and hence the charge pattern are only marginally perturbed by such alterations to the ionic concentration. This makes it possible to probe for repulsive and attractive domains within a variety of myosin-based structures. Using this approach in conjunction with the constraints imposed by the kinetics and thermodynamics of synthetic filament assembly, we have investigated the structural basis for the length regulation mechanism as mediated by the myosin molecule in thick filament assembly.

Structural Evidence for Macroscopic Domains

Since the extent of interactions between charged domains can be amplified by varying the ionic strength from moderate concentrations of salt (0.15 M) at which filaments are generated to low concentrations (0.01 M), it is possible to probe for predominantly repulsive and/or attractive domains in thick filaments and to physically identify the resultant structural changes by electron microscopy.

Native filaments isolated from vertebrate skeletal muscle split into three subfilaments under conditions of low ionic strength (12, 13). Splitting is limited to the two regions of parallel-packed myosin; the central bare zone appears to remain intact. This organization of myosin subunits into three subfilament-like zones is not only

observed in thick filaments treated with low ionic strength solvents; it has been seen in various transverse sections of native filaments (16, 17). The individual subfilaments are considered to be formed from parallel-packed myosin alone (17). They extend into the region of antiparallel overlap, where they coexist with subfilaments of opposite polarity (17). The point in the structure at which thick filament splitting ceases has yet to be precisely located.

Assumably, in light of the high degree of structural equivalence, synthetic filaments formed from vertebrate skeletal myosin between pH 8.0 and 8.5 share this basic underlying structure with native thick filaments. Lowering the ionic concentration of pH 8.0 filaments causes these filaments to shorten in length rather than split into subfilaments; minifilaments (discussed later) represent a limit product, under the experimental conditions used, for this process. Subfilaments have been seen in preparations of the pH 7.0 class of synthetic thick filaments (18). These filaments are considered to be similar to pH 8.0 filaments, but have additional myosin molecules added circumferentially (19).

The heterogeneity in the structure of the filament implies that assembly occurs through a sequential mechanism in which at least two types of subunit interactions participate. The stable subfilaments appear to be formed from strong attractive ionic interactions, with weaker intersubfilament domains holding the three subfilaments together. The existence of strongly repulsive intersubfilament domains is deduced from the simple observation that the thick filament would not split were they absent, and not coupled to the weak intersubfilament interactions. It is therefore proposed that the weak and the repulsive intersubfilament interactions are additively coupled to each other through the rigidity within each of the three subfilaments. There is, unfortunately, no direct measure of the rigidity of these subfilaments; however a notable characteristic of short synthetic filaments and native filaments is that they generally appear as linear, rigid structures. Since the repulsive interactions appear to be masked specifically in certain parts of the native thick filament by proteins carrying a complementary charge, it seems likely that the weak and repulsive interactions are located separately on the subfilament surface. Masking, or the neutralization of the repulsive component, is observed in those parts of the thick filament that fail to split into subfilaments. The bare-zone region provides one example; the other is seen at the tips of the three subfilaments that appear to be held together by end-filaments (13), i.e., nonmyosin structures located centrally at the tips of native thick filaments. Therefore, the repulsive component appears to operate between like charges, in all probability, positioned on the subfilament surfaces at or close to the helix axis. The repulsive charges are almost certainly negative, originating from carboxyl groups, since an increase in the pH of the medium shortens pH 8.0 synthetic thick filaments (20). This could arise from a weakening of the attractive interactions as the cationic acid component of the ion pairs progressively deprotonates, whereas the repulsive contribution arising from charged neutral-acid groups remains constant. The net result is that the negative cooperativity per unit length would rise more sharply under alkaline conditions causing assembly to cut off earlier with the consequent formation of shorter filaments. A schematic representation showing the major features of the proposed model is provided in Fig. 1.

Interpretation of the Kinetic and Thermodynamic Data

The mechanism has to be consistent with the results of the kinetic experiments in which a cumulative property progressively destabilizes the structure of the dimer binding site as the filament grows in size (2, 4); specifically, with a linear increase in Gibbs energy coupled to a simultaneous and linear decline in the magnitude of attractive electrostatic interactions in the binding of dimer at the tip of the thick filament (4). These linear dependencies place definite constraints on the physical basis of the length-regulation mechanism. To illustrate how this type of behavior might arise, the interaction of two opposing domains that carry a number of identical, uniformly distributed charge loci is considered: here, the overall Gibbs energy for the interaction between the two domains is proportional to the number and strength of the interacting charge pairs. As each charge pair is assumed to be identical, the total Gibbs energy for domain-domain interaction would be directly proportional to the number of pairs of interacting charges. The growth of such a domain would give rise to linear dependencies between the Gibbs energy, the extent of charge-charge interaction, and the

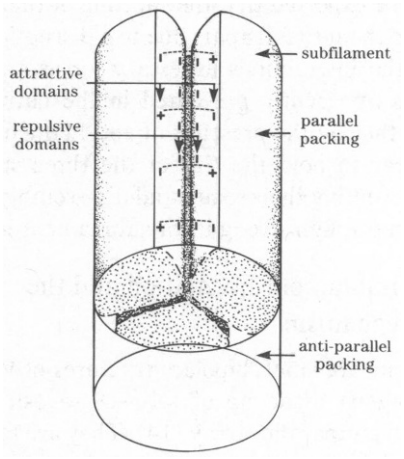

FIGURE 1 Schematic representation of the main features of the model for length regulation in a segment of structure located about the interface of the antiparallel and parallel-packed zones of the thick filament. The boundary between parallel and antiparallel-packed myosin was selected for illustrtion for the sake of simplicity. It is likely that the length regulation mechanism based on the repulsion between parallel-packed subfilaments extends some distance into the region perturbed by antiparallel overlap.

size of the structure. This additive relationship between attractive and repulsive charge-charge interactions and the overall Gibbs energy is equivalent to the situation observed experimentally with myosin filament assembly.

The strictly sequential (the serial addition or loss of dimer subunit) nature of myosin filament assembly (2, 4) places further limitations on the mechanism. For the process of assembly to occur, all the various interactions in the sequential series have to be favorable. The introduction of a predominantly repulsive interaction would cause assembly to terminate. This is an additional reason for having chosen to localize the repulsive and attractive intersubfilament interactions in physically separate domains with the weak interactions coupled to the repulsive component. Assembly of parallel-packed myosin can only initiate under circumstances in which the positive interactions are stronger than the repulsive component. Physically, this might arise in a zone with structural heterogeneity, for example, at or close to the interface of the parallel and antiparallel regions of the thick filament. This could explain why segments of parallel-packed myosin do not spontaneously nucleate and grow to produce monopolar filaments. The contribution of the repulsive component would thereafter have to increase at a faster rate than the positive interactions. A coupling of these processes to each other would ultimately lead to the termination of growth at the point where the positive interactions in the dimer binding site had been weakened (solvated) to the extent that a dimer-filament equilibrium was established. In light of the potential heterogeneity of dimer binding sites at the tip of the thick filament, it would only be those sites with a preponderance of weak interactions coupled to the repulsive component that regulate length.

It might be expected that the splitting of thick filaments would cause them to fall apart due to a disruption of one of the sequential interactions necessary for assembly. However, this is apparently prevented in the native filament structure either by the presence of end-filaments (12, 13), which appear to hold the tips of the three subfilaments together preventing their sequential disassembly and/or by stabilization through strong intra-subfilament bonds.

Minifilament Formation and the Mechanism

Minifilaments are small bipolar structures of well-defined length (0.3 μm) made up of ~16–18 myosin molecules packed in an antiparallel array (14). They are formed from myosin by dialysis against a solution of low ionic strength and pH similar to that used to split native filaments. Structurally, they are believed to be an intermediate formed on the synthetic filament assembly pathway; since, on raising the ionic concentration, minifilaments are transformed into pH 8.0-type synthetic filaments (15). The experimental evidence seems to support a mechanism in which a single minifilament grows into a thick filament by the addition of subunit rather than by the lateral aggregation of minifilaments before growth. At low ionic concentrations, these small filaments are incapable of growing into synthetic filaments by the addition of myosin to form the parallel-packed arms. It seems reasonable to propose that the weak intersubfilament bonds are unstable in the presence of the strongly enhanced repulsive component, and as a result, the sequential process of assembly into synthetic filaments is disrupted. Their length corresponds roughly to the inner limit of the dissociation kinetics typical of the parallel-packed arms of the thick filament (4).

In view of the nature of the ionic bonding in the parallel and antiparallel packed regions of synthetic myosin filaments, clearly the length of minifilaments is precisely controlled (4). A combination of the length curtailment mechanism described above and one other property ensures stability. As was mentioned earlier, the electrostatic component of the favorable interaction between the subunits in a segment of synthetic thick filament roughly equivalent in size to a minifilament, increases with size toward a maximum at a point close to the boundary of the antiparallel and parallel packed myosin (4). The remarkably precise length of minifilaments is, consequently, the product of a discontinuity between these highly favored attractive ionic interactions and strong repulsive ionic interactions characteristic of parallel-packed material.

CONCLUSION

The length regulation mechanism discussed above highlights the importance of ionic bonding in the determination of the size and structure of complex polymers. The mechanisms of size regulation in mini- and synthetic filaments appear to be primarily directed by changes in the charge-charge interactions with aggregate size. In the former case it results in a remarkably uniform length, controlled by a mechanism in which structural discontinuity plays an important role; with the latter, in a narrow-length distribution in an aggregate of essentially uniform structure. In native filament assembly it is difficult to formulate a simple and explicit role for ionic interactions because of the inherent complexity of an assembly mechanism that involves the interaction of numerous co-polymerizing components that function as modifiers of the underlying myosin-mediated mechanism. However, recent experimental results on the kinetics of native filament dissociation in muscle fibers have shown an exponential dependence of the rate of dissociation on filament length (21), similar to that found in the earlier kinetic experiments on synthetic filament dissociation (2, 4). This finding strongly supports the author's contention that the myosin-based assembly mechanism formulated from the kinetic studies plays a fundamental role in native filament assembly. A fine tuning of the myosin-mediated mechanism by external

information in the form of co-polymerizing proteins and/or covalent modification would give rise to the precise length of the native filament. These interactions are not resolved in the fiber dissociation experiments mentioned above; only the kinetic signature of the myosin-mediated component of the reaction is observed.

Of the models proposed for the physical basis of length regulation in biopolymers and for thick filament assembly, the Vernier model (22) has received much attention. It is therefore worthwhile to ask if its characteristic features can be detected in filament assembly from purified myosin. The model would require that co-linear subfilaments, assembled via a sequential mechanism, shift gradually out of register, thereby increasing the Gibbs energy of subunit interaction at the growing tip to the extent that assembly is terminated. It is conceivable that the three subfilaments might, through some perturbation of their structure, function in this way. If this were indeed the case, the Gibbs energy of interaction of filament with subunit would rise in parallel with the reciprocal of the distance separating the interacting charges, but the assembly of parallel-packed myosin is characterized by a linear relationship between Gibbs energy and length (2, 4). This behavior is consistent with our charge-based model and not with a Vernier model. A Vernier mechanism could potentially play a role in native filament assembly. The myosin part of the thick filament structure could move out of register with the subunit repeat of a co-polymer component and thus regulate size.

The interaction of the repulsive component with a centrally-located, length-regulating protein within the structure of the native filament of vertebrate skeletal muscle, though an attractive possibility, seems unlikely on examination of the available evidence. Cleavage of the filament into three subfilaments would not happen if a protein of complementary charge ran the length of the core of the parallel-packed region. This is a different situation to that encountered in the concentric mode of assembly of invertebrate thick-filaments in which myosin surrounds a paramyosin layer formed about a proposed "ruler-like" central core, in which such a mechanism does presumably operate (23). Evidence of the disruption of thick filament assembly in vertebrate skeletal muscle by interaction with a protein of complementary charge is limited to the filament ends. A bulky protein bound at the center of the tip of the filament could disrupt the sequential process of assembly in a precise and abrupt manner. End-filaments (13), structures that remain attached to the tips of the native filament under conditions of exaggerated charge-charge interaction, are likely candidates for this role. How these end-filaments come to be located at 1.6 μm is a matter on which little useful comment can be made at this stage.

I would like to thank Dr. William F. Harrington for comments on the manuscript.

The work was supported by grants from the National Institutes of Health (AM-04349 to W. F. H.) and the Vice-Chancellor's Research Award of the Witwatersrand University. This is contribution No 1318 from the Department of Biology, Johns Hopkins University.

Received for publication 3 October 1985 and in final form 31 March 1986.

REFERENCES

1. Davis, J. S. 1981. The influence of pressure on the self-assembly of the thick filament from the myosin of vertebrate skeletal muscle. *Biochem. J.* 197:301–308.
2. Davis, J. S. 1981. Pressure-jump studies on the length regulation kinetics of the self-assembly of myosin from vertebrate skeletal muscle into thick filament. *Biochem. J.* 197:309–314.
3. 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.
4. Davis, J. S. 1985. The kinetics and thermodynamics of the assembly of the parallel and antiparallel packed sections of synthetic thick fialments of skeletal myosin: a pressure-jump study. *Biochemistry.* 24:5269–5275.
5. Davis, J. S. 1981. Myosin filament self-assembly: a new model for supramolecular structure generation. *S. Afr. J. Sci.* 77:499–500.
6. Davis, J. S. 1983. Myosin filament assembly and supramolecular structure generation. *Biophys. J.* 41(2, Pt. 2):299*a*. (Abstr.)
7. Caspar, D. L. D. 1963. Assembly and stability of the tobacco mosaic virus particle. *Adv. Protein Chem.* 18:37–118.
8. Kellenberger, E. 1969. Polymorphic assemblies of the same major virus protein subunit. *In* Nobel Symposium II. A. A. Engstrom and B. Strandberg, editors. John Wiley & Sons, New York. 349–366.
9. Wagenknecht, T., and V. A. Bloomfield. 1975. Equilibrium mechanisms of length regulation in linear protein aggregates. *Biopolymers.* 14:2297–2309.
10. Kensler, R. W., and M. Stewart. 1983. Frog skeletal muscle thick filaments are three-stranded. *J. Cell Biol.* 96:1797–1802.
11. Josephs, R., and W. F. Harrington. 1968. On the stability of myosin filaments. *Biochemistry.* 7:2834–2847.
12. Maw, M. C., and A. J. Row. 1980. Fraying of A-filaments into three subfilaments. *Nature (Lond.).* 286:412–414.
13. Trinick, J. A. 1981. End-filaments: a new structural element of vertebrate skeletal muscle thick filaments. *J. Mol. Biol.* 151:309–314.
14. Reisler, E., C. Smith, and G. Seegan. 1980. Myosin minifilaments. *J. Mol. Biol.* 143:129–145.
15. Reisler, E., P. Cheung, C. Oriol-Audit, and J. A. Lake. 1982. Growth of synthetic myosin filaments from myosin minifilaments. *Biochemistry.* 21:701–707.
16. Stewart, M., F. T. Ashton, R. Lieberson, and F. A. Pepe. 1981. The myosin filament. IX. Determination of subfilament positions by computer processing of electron micrographs. *J. Mol. Biol.* 153:381–392.
17. Luther, P. K., P. M. G. Munro, and J. M. Squire. 1981. Three-dimensional structure of the vertebrate muscle A-band. III. M-region structure and myosin filament symmetry. *J. Mol. Biol.* 151:703–730.
18. Pinset-Härström, I. 1985. MgATP specifically controls in vitro self-assembly of vertebrate skeletal myosin in the physiological pH range. *J. Mol. Biol.* 182:159–172.
19. Kaminer, B., and A. B. Bell. 1966. Myosin filamentogenesis: effects of pH and ionic concentration. *J. Mol. Biol.* 20:391–401.
20. Josephs, R., and W. F. Harrington. 1966. Studies on the formation and physical chemical properties of synthetic myosin filaments. *Biochemistry.* 7:2834–2847.

21. Higuchi, H., and S. Ishiwata. 1985. Disassembly of thick filaments in rabbit skeletal muscle fibers. Effects of ionic strength, Ca^{2+} concentration, pH, temperature and cross-bridges in the stability of thick filament structure. *Biophys. J.* 47:267–275.
22. Huxley, H. E., and W. Brown. 1967. The low-angle x-ray diagram of vertebrate striated muscle and its behavior during contraction and rigor. *J. Mol. Biol.* 30:383–434.
23. Epstein, H. F., D. M. Miller, I. Oritz, and G. C. Berliner. 1985. Myosin and paramyosin are organized about a newly identified core structure. *J. Cell Biol.* 100:904–915.