Review

Myosin Heads and Thick Filaments: Recent and Older Experiments and Hypotheses

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Review

Myosin Heads and Thick Filaments: Recent and Older Experiments and Hypotheses

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Abstract

The organization and roles of the two heads of myosin in thick filaments from skeletal muscle remain unresolved. Here, I try to reconcile the various points of view. I recommend avoiding comparisons of skeletal muscles from vertebrates (e.g. frog, rabbit, mouse) with those from invertebrates (e.g. tarantula, a spider) and insect flight muscles (IFM; e.g. from *Lethocerus*). Animal age should also be taken into account, as demonstrated in studies of the contractile properties of isolated skeletal muscles and permeabilized fibers from young, adult and old mice. The myosin content of natural filaments from old and very old rabbits (i.e. animals weighing ~ 5-7 kg) is lower than that of young adult rabbits (i.e. animals weighing ~ 2-3 kg), and their myosin molecules are arranged differently (two strands in older rabbits, three in younger animals). Sarcopenia — the loss of specific contractile force during aging, in particular — can be explained at least partly by the decrease in myosin content (- 1/3) of natural thick filaments. Moreover, specific MgATPase activity per myosin molecule (measured in vitro, under resting conditions, in natural thick filaments) is lower in old than in young rabbits. These two experimental observations probably account for a significant proportion of sarcopenia.

Keywords: myosin heads; S1; thick filaments; aging; sarcopenia

1. Introduction

Over the last 45-50 years, one question frequently posed in the domain of vertebrate skeletal muscle relates to the existence and roles of the two heads of myosin (also called myosin II). This major problem was evoked as early as 1974 by Sir Andrew Huxley [1], and the existence and roles of the two heads remain puzzling [2–4]. Here, I provide an analysis and interpretation of the situation in which I try to reconcile old neglected experiments, hypotheses, and discussions with new experimental discoveries and interpretations concerning the molecular structure of the thick filaments. In this context, I am following the same general line as Irving [5] and Taylor et al. [6], who have also stated that the structure and function of the thick filaments remain a matter of concern. However, the approach used by my group, based on various experimental techniques and presented over a period of about 20 years, differs from that of these authors, and is probably complementary to their approaches.

2. Complex Problems and More Confusion

Many experiments, using a spectrum of techniques, have been performed on different types of muscle (healthy or diseased). Multiple explanations and interpretations have been proposed for the characteristics of the thick filaments, and for the properties and possible roles of the two myosin heads, under contraction or relaxation conditions in vivo/in situ and also in vitro [7–23]. Many of these studies are complex and may not be entirely satisfactory. However, their conclusions remain plausible, with some considered beyond dispute by most specialists in the field of muscle and muscle contraction. This is the case, for example, for the gradual development (from about 2010 to 2025) of

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the notion of the "interacting-heads motif - IHM", which probably resembles, in situ, the "OFF-state" crossbridges unable to bind to thin actin filaments, and that of the perhaps related "super-relaxed state - SRX" (for more details, see [24–29]). There is widespread agreement about the IHM and the SRX state and their relationship, but I have my doubts, because I think that an alternative and complementary approach to relaxation and to most of the swinging crossbridge/lever-arm mechanisms of muscle contraction in vivo/in situ is possible, provided that another structure is proposed for the thick filaments (see Section 3). Caremani et al. [30] concluded from their experiments that the conclusion drawn from observations of the IHM is probably an oversimplification. Over a period of about 20 years (between 1980 and 2001), my group tried to find appropriate and simple explanations for the existence of the two heads of myosin II and their roles in vertebrate skeletal muscles. I propose here a reexamination of the situation, based particularly on experimental findings and discussions largely neglected by the muscle community.

3. Another Approach to the Roles of the Myosin Heads in the Arrangement of Thick Filaments from Vertebrate Skeletal Muscles

We [31] used analytical ultracentrifugation at 4°C to show that the soluble myosin S1 heads (myosin prepared from young adult rabbit skeletal muscles) could form dimers in conditions resembling those of the sarcoplasmic medium (monomer-dimer mixtures in rapid reversible equilibrium). Based on this finding, we [32] put forward a hypothesis concerning the contribution of the S1 head dimer to the characteristics of thick filaments (see the last paragraph of this section). It has been claimed that the experimental study of Hu et al. [10], for example, is not consistent with our 1982 hypothesis. These authors used cutting-edge techniques to describe in detail the structure of thick filaments from relaxed *Lethocerus* flight muscle (IFM), without citing our hypothesis. I would argue that it is risky to claim that the study of Hu et al. [10] disproves our 1982 conclusions, for at least three reasons:

(i) Paramyosin is frequently (possibly always) present in the core of insect flight muscle thick filaments and also in the core of tarantula thick filaments — highly informative papers on this subject have been published [33–35] — and it is generally impossible for "internal" myosin heads to exist. This is a fundamental difference with respect to the thick filaments of vertebrate skeletal muscles. Comparing thick filaments of various origins, Miller et al. [36] provided a clear qualitative and quantitative description of the presence of paramyosin and many other components.

(ii) Most authors (e.g. [10]) did not compare the estimated number of heads lying outside the backbone with the expected number, a fundamental parameter that we studied in our 1982b paper [32] - see the second part of the last paragraph of this section.

(iii) The age of the insects studied is unknown (another potentially important parameter - see the end of the last paragraph of this section).

I conclude from this short analysis that the two types of thick filaments (from IFM and vertebrate skeletal muscles) are not comparable. This point of view is supported by Levine [37], who demonstrated that the arrangement of myosin heads on relaxed thick filaments differs considerably between *Lethocerus* and rabbit muscles[1]. Insect flight muscles can relax and contract without difficulty because all heads lie outside the backbone and can work (e.g. swinging crossbridge/lever-arm mechanisms - see Section 4 regarding the contribution of titin to contraction), as do all the "external' heads in vertebrate skeletal muscles. In vertebrate skeletal muscles about 100% - 40% = 60% of the isometric tetanic force is related to the "external" heads (see Section 4), whereas the equivalent percentage for insect flight muscles is probably about 100% because all the heads lie on the external surface of the thick filaments, as in tarantula muscles. It should be stressed that, in traditional models, the swinging crossbridge/lever-arm mechanism is probably insufficient to account for the full isometric tetanic force, at least in skeletal and cardiac muscles (see again Section 4 concerning the complexity of the interfilament medium, particularly the presence of the "active" giant protein titin).

The discovery of the S1 head dimer was contested in 1986 (unpublished results) and then violently rejected by the muscle community. Over the next decade, we resolved the origins of this

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conflict: the S1 dimerization site is extremely labile and at least 16 experimental criteria must be respected to ensure that it remains intact and functional [38]. A 17th criterion should also be added: the age of the rabbit. I have doubts about the presence of the dimerization site on S1 prepared from the muscles of very old rabbits. Despite the internal cleavages in S1, resulting from the controlled digestion of myosin [39], isolated S1 and the intact heads of intact myosin behave similarly, as demonstrated by my group. Only a few quantitative differences would be expected, and the possible frailty of the dimerization site of "very old" S1 is probably not observed in "very old" myosin heads. The S1 dimer and myosin head-head dimer are important because they are involved in the arrangement of the thick filaments of skeletal muscles, as recalled below (see in particular the paragraph concerning our 1999 article).

Bachouchi et al. [40,41], Grussaute et al. [42] and Morel and Guillo [43] used various techniques (e.g. freeze-fracture-EM in solution, MgATPase activity assays, viscometry, analytical and preparative ultracentrifugation, fluorometry) to confirm the existence of the S1 dimer in solution, and to describe some of its biophysical and enzymatic properties. Using biochemical techniques, Kuntz et al. [44] and Schaub et al. [45] were probably the first to detect head-to-head interactions in isolated myosin. Winkelman et al. [46] obtained crystals of S1 from avian muscles and observed, by EM and reconstruction techniques, that the elementary motif of the crystals was an S1 dimer (not highlighted by the authors but clearly observed in certain figures). Using the freeze-fracture, deep-etch, rotary shadowing technique (EM) on stretched frog muscle fibers (no overlap between the thin and thick filaments), Suzuki and Pollack [47] observed bridge-like interconnections between the neighboring thick filaments, which they attributed to myosin head-to-head dimers (see also [48], for supporting experiments). Using traditional EM (negative staining) methods, Podlubnaya et al. [49] observed ordered assemblies of myosin minifilaments, resulting from head-head interactions between different minifilaments. Claire et al. [50] used physical techniques (e.g. polarized dynamic light scattering and MgATPase activity assays) to confirm the existence of the S1 dimer in solution. Using light scattering (at 90°, wavelength 500 nm), we [16] observed numerous head-head interactions between filaments in the presence of various concentrations of MgATP (which promotes S1 dimerization). Unfortunately, all these experimental results were, and are still, largely ignored by the muscle community. However, the notion of an "interacting-heads motif-IHM" on thick filaments may be related to the head S1 dimer.

We published two articles [38,51], based on old and more recent techniques (e.g. viscometry, laser light scattering, high performance capillary electrophoresis HPCE), confirming the results we obtained in 1982, 1985, 1986 and 1995 for S1 dimerization, and extending this property to soluble myosin (at high ionic strength - via its two heads).

We then published a detailed experimental article [16] confirming and refining the hypothesis put forward in 1982 [32], through diverse experimental techniques (e.g. negative staining-EM, light scattering, controlled digestions, MgATPase activity assays). We, thus, demonstrated that myosin head S1 dimerization plays a fundamental role in synthetic and natural (native) thick filaments in vitro. All our biological material was prepared from adult rabbit skeletal muscles. More precisely, we found that one of the two heads is, indeed, inserted into the core of the thick filament, where it forms a dimer (of the S1-S1 type) with the internal head of the diametrically opposite myosin molecule. The second head lies outside the filament backbone and forms the major part of a cycling crossbridge during contraction. Diagrams of this arrangement can be found in two of our papers [16,51]. It should be stressed that the "internal" heads clearly stabilize the thick filaments in the axial and radial directions in a manner similar to that involving paramyosin in certain muscles. Several groups have shown that the thick filaments of frog skeletal muscles are three-stranded (e.g. [13,52]). A similar architecture has been reported for the thick filaments of rabbit skeletal muscles and, frequently, but not systematically, the muscles (skeletal, cardiac and smooth) of other animal species (e.g. [14,19])[1]. In our 1999 paper [16], we confirmed that the natural thick filaments of young adult rabbits (psoas muscle) are three-stranded, but the arrangement of the heads recalled above relates to only two of the strands. In a sarcomere, the heads of the myosin molecules on the third strand may be arranged

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as suggested by Offer and Elliott [17]. This hypothesis is valid for insect flight muscles (IFM) and can be extended to skeletal muscles, with minor modifications concerning the shape and length of the myosin heads in particular [53,54]: the two heads corresponding to the third strand lie outside the shaft of the thick filament and could interact with two different neighboring actin filaments. The problem of actin-myosin interactions, thus, has many facets due, in particular, to the existence of the two heads . In this context, Wu et al. [20] demonstrated in a study of insect flight muscles (IFM) that the binding to the thin actin filaments of the two-headed myosin molecules during isometric contraction is highly complex. This study raised questions about the extent to which the discoveries of these authors also apply to vertebrate skeletal muscles (see, e.g. first paragraph of this section for comments on IFM and skeletal muscles). Whatever the exact position of the heads and the various geometric constraints for binding to the thin filaments in a sarcomere [53,55,56], concerning major geometric constraints, even near the slack length of a fiber, and under weak external osmotic pressure), in our model, the two "external" heads, belonging to the same myosin molecule and located on the third strand, are involved in the traditional swinging crossbridge/lever-arm process during contraction (see second paragraph of this section and Section 4, regarding this traditional process), and may be involved in the IHM and SRX state, mostly in resting conditions. In our proposed structure, which is valid at least for frog and rabbit skeletal muscles, ~ 200 heads are inserted within the thick filament core and ~ 400 lie outside it [57]. The late Gerald Offer grew increasingly dubious about the hypothesis he had previously put forward with Elliott [17][2]. Assuming that this model is no longer valid, the two "external" heads corresponding to the third strand, and the "external" head corresponding to the "internal head" (total of ~ 400 heads) can participate in the IHM observed on thick filaments from various muscles (see Section 2). The difference between ~ 600 external heads (traditional view, all heads outside the filament core) and ~ 400 external heads (our model) is almost certainly too small for it to be concluded that our approach is unrealistic, owing particularly to the major uncertainty on the number of heads highlighted in point (ii) in the first paragraph of this section. In 1999, we also studied old rabbits (psoas muscle) and found that the natural thick filaments of these animals had only two strands (see also 57). In this context, the hypothesis of Offer and Elliott [17] is not useful. The above description of two-stranded filaments applies, and there are ~ 200 "internal" and only ~ 200 "external" heads (total of ~ 400 heads). The relative loss of ~ 100 x (600 - 400) heads/600 heads ~ 33% of the myosin content of a sarcomere almost certainly makes a major contribution to the many-faceted phenomenon of sarcopenia (e.g. loss of specific active force during aging in humans, rabbits, and possibly other animal species - see Abstract and Appendix for further details concerning the possible molecular origins of sarcopenia). Many articles and books on sarcopenia have been published [58,59]. Many groups have studied the many-faceted problem of aging [60–72], which is nevertheless ignored by too many specialists in muscle and muscle contraction. The IHM and SRX state occur mostly in conditions of relaxation, and a value of ~ 33% at rest could be concealed by the ~ 20-25% margin of error estimated in Appendix 5.I of my 2015 book [57].

4. Discussion

In 2015, I published a monograph on muscle contraction [57]. This treatise presents a new analysis of many published findings, together with previously unpublished experimental results largely confirming the importance of myosin head (S1) dimerization for the structure of thick filaments from vertebrate skeletal muscles in vivo/in situ[3]. It is shown that these filaments make a major contribution to contraction, accounting for ~ 40% of the isometric tetanic contractile force, through previously unsuspected processes. This book may provide answers to the questions posed some months before its publication by Herzog et al. [71] and Månsson et al. [4], who considered the molecular processes of muscular contraction to be largely unknown. At this point, it should be recalled that the elongated giant protein titin (MW ~ 4 MDa) — present in all sarcomeres of striated skeletal and cardiac muscles (at least those from certain vertebrates), tightly associated with the thick filaments, and located between the Z discs and the M lines — almost certainly plays a role in both

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resting and contracting muscles. This adds an additional layer of complexity to our understanding of both resting and contraction conditions [73–96]. Moreover, Daneshparvar et al. [2,3] have compared thick filaments from *Lethocerus* and *Drosophila melanogaster*. They concluded that the myosin heads of the thick filaments of *Drosophila*, unlike those in *Lethocerus*, are disordered and that it is uncertain whether there is an interacting heads motif (IHM). In any event, as things stand, I would suggest that the IHM-SRX state may be at least partly hindered in vivo/in situ, because all the myosin heads are assumed to lie outside the filament core and would therefore compete with titin. A much smaller protein, myosin-bound protein C (MyBP-C or cMyBP-C - MW ~ 140 kDa), is also anchored to both myosin and titin, and plays a role in the interactions between the myosin heads and actin filaments [97–102]. By contrast, in our approach to the thick filaments of skeletal muscle, the "internal" heads do not interact with the proteins lying on the outer surface of the filaments (e.g. titin).

5. Conclusion

The traditional IHM has frequently been described in studies on isolated thick filaments (using EM techniques), whereas the SRX state has been described in studies on whole demembranated fibers (in situ)[4]. In this section, I will deal with some of the salient points I raised in a recent publication [103]. Concerns about macromolecular crowding arise for whole fibers, but not isolated single thick filaments. This issue is well covered by Ellis [104] and Minton [105,106], and, more specifically, by Ge et al. [10]). The importance of studying the IHM and SRX state, and other complex phenomena under in situ conditions, is illustrated by the work of Caremani et al. [108], who published an experimental study of the characteristics and properties of thick filaments in relaxed mammalian skeletal muscle (demembranated fibers and intact muscles), under various conditions of temperature and interfilament spacing (controlled by the lateral osmotic compression of demembranated fibers). These authors highlighted the increasing complexity of the interfilament medium (e.g. presence of titin, MyBP-C, nebulin, obscurin – [109–114]; the existence of "sarcoplasmic/cell-associated water", different from bulk water, should also be taken into account - see [115–118])[5], with possible structural and functional consequences for thick filaments (see also the preceding paragraph). It should also be borne in mind that thick filaments in situ (i.e. in an aqueous medium at pH ~ 7) contain many negative fixed charges and bound anions [119–123], whereas these filaments are electrically neutral when observed by EM, due to the dry environment (with the exception of certain specific EM techniques [124–127], not used by the authors cited here)[6]. These findings almost certainly constitute additional major stumbling blocks, likely to generate different and possibly contradictory conclusions. The IHM-SRX state hypotheses therefore raise a number of concerns ([103,128,129] for experimental studies and critical analyses), but none of the models proposed to date is universal – in [103], the symbol** at the end of the first paragraph of the introduction relates to [128] and not [129]).

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APPENDIX

For natural thick filaments (prepared from rabbit psoas muscles, at a physiological MgATP concentration (3 mM), the "resting" MgATPase activities [at (20.0 ± 0.1)°C] are ~ 0.0225 s^{-1} for thick filaments from young adult rabbits (4 months old) and ~ 0.0119 s^{-1} for thick filaments from old rabbits (18 months old) (16). Thus, old rabbits have an MgATPase activity relative to young adult rabbits of ~ 100 x (0.0119 s^{-1}/ 0.0225 s^{-1}) ~ 53%. I recall in the abstract that the relative myosin content of the thick filaments of old rabbits is ~ (1 - 1/3) = 2/3, relative to young adult rabbits. The "resting" MgATPase

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activity for "old thick filaments" would be ~ 53% x 2/3 ~ 35% that for "young thick filaments". This lower estimate of the "resting" MgATPase activity for old rabbits than for young adult rabbits would be different under contraction conditions. Moreover, there is no straightforward relationship between MgATPase activity under contraction conditions and the force developed, regardless of the model of contraction. Nonetheless, in future experimental studies of aging and sarcopenia, it would be interesting to measure the MgATPase activities of old muscles for comparison with those of young muscles.

Notes

1. In this context of complexity and confusion, it should be noted that other major properties of thick filaments differ between animal species. As early as 1994, Kensler et al. [130] showed, by EM, that the crossbridge order in isolated thick filaments from fish is similar at 4°C and 25°C (to my knowledge, the same is true for frog), whereas the crossbridge order in rabbits is similar to that in fish at 25°C, but not at 4°C.
2. Wang et al. [131] studied the organization of mouse psoas sarcomeres in rigor and concluded that the two heads of a myosin molecule mostly bind to two neighboring actin subunits in a thin filament, but that the arrangement proposed by Offer and Elliott [17] is possible for some myosin molecules.
3. Using light scattering (at 90°, wavelength 500 nm) on suspensions of synthetic thick filaments from young adult rabbit, at various temperatures (between 35 and 40°C), whether increasing or decreasing, I also found that one head of the myosin molecule is inserted into the core of the synthetic thick filament and reversibly associates with and dissociates from the internal head of the diametrically opposite myosin molecule (internal head-head dimer - see Chapter Five, in particular Section 5.2 - in Appendix 5.II, I conclude that contractures/cramps in whole muscles are closely related to this observation - the old "lactic acid dogma" is no longer valid, because demembranated fibers undergo fatigue during long tetani: see Chapter Seven). Note also that synthetic thick filaments fray rapidly and reversibly into two subfilaments [132] and are almost certainly two-stranded.
4. Most experimental studies on the SRX state have been performed on glycerinated fibers. As I recently pointed out [103], this process can lead to erroneous results (relative to removal of the membrane barrier by mechanical skinning or soft chemical permeabilization). This opinion is exemplified by Bartels and Elliott [133] and Millman [134].
5. Chu et al. [135], investigated the possible existence of the IHM and the SRX state in solution (on soluble heavy meromyosin, HMM – a two-headed myosin subfragment prepared from cardiac myosin). The many results presented require a complex interpretation, but I note that, in most cases studied, the IHM and the SRX state are difficult to identify and their relationship is not straightforward and may even be considered weak in certain conditions. This leads me to wonder whether the IHM and SRX state described by Chu and coworkers are really similar to the 'traditional" IHM and SRX state [103].
6. Gollapudi et al. [136] studied the possible existence of the SRX state on synthetic thick filaments (prepared from cardiac myosin). The synthetic filaments, in suspension in various buffers, undoubtedly contained many fixed negative charges and bound anions. However, the problem of crowding has yet to be resolved. Moreover, the authors used an unconventional technique to prepare synthetic thick filaments. It would be interesting to perform similar investigations with the slow dilution process (see [16,132,137]).

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