Journal of
Nanomedicine & Nanotechnology

Sugi and Chaen, J Nanomed Nanotechnol 2016, 7:3
DOI: 10.4172/2157-7439.1000377

Review Article Open Access

Evidence against the Swinging Lever Arm Mechanism in Muscle Contraction Based on the Effect of Antibodies to Myosin Head

Sugi H[1]* and Chaen S[2]

[1]Department of Physiology, School of Medicine, Teikyo University, Tokyo, Japan
[2]Department of Integrated Sciences in Physics and Biolohy, College of Humanities and Sciences, Nihon University, Tokyo, Japan

Abstract

During muscle contraction, myosin heads extending from myosin filaments first attach to actin filaments, perform power stroke producing force and motion in muscle, and then detach from actin filaments. A myosin head (or myosin subfragment-1, S-1) consists of catalytic and lever arm domains (CAD and LD), which are connected via converter domain (COD). It is widely believed that the myosin head power stroke is caused by swinging lever arm mechanism, which assumes active rotation of the LD around the COD, caused by structural changes in and around the COD. The lever arm mechanism is, however, constructed from nucleotide-dependent structural changes of crystals of truncated myosin head, consisting only of the CAD and the COD, and therefore overlooks possible role of the LD and myosin subfragment-2 (S-2), connecting myosin heads to myosin filament backbone. In this article, we present evidence against the lever arm hypothesis based on the following results: (1) antibody to reactive lysine residue (Lys83) located in the COD (anti-RLR antibody) has no effect on Ca^{2+}-activated muscle fiber contraction; (2) using the gas environmental chamber attached to electron microscope, we record ATP-induced power stroke of myosin heads, position-marked with anti-RLR antibody; (3) antibodies to myosin head LD (anti-LD antibody) and to myosin S-2 (anti-S-2 antibody) inhibit Ca^{2+}-activated contraction without changing MgATPase activity. The absence of inhibitory effect of anti-RLR antibody, attaching to RLR in the COD, makes the lever arm mechanism unlikely, since attachment of bulky antibody (IgG) to RLR in the COD is expected to inhibit structural changes in and around the COD. Meanwhile, inhibitory effect of anti-LD and anti-S-2 antibodies indicate essential role of the LD and the S-2 in muscle contraction.

Keywords: Muscle contraction; Myosin head power stroke; Lever arm mechanism; Antibodies to myosin; Gas environmental chamber; Myosin subfragment-2; X-ray diffraction

Introduction

In 1954, Huxley and Hanson [1] and Huxley and Niedergerke [2] made a monumental discovery that muscle contraction results from relative sliding between two kinds of myofilaments, i.e., myosin and actin filaments [1]. As shown in Figure 1A, a myosin molecule (MW 450,000) is enzymatically split into two parts; a rod (light meromyosin, LMM) of 113 nm long and the rest of the molecule (heavy meromyosin, HMM), with two pear-shaped heads (myosin subfragment-1, S-1) and a short rod (myosin subfragment-2, S-2). In muscle, LMM aggregates to form myosin filament backbone, while myosin S-1 heads extend laterally from filament backbone with an axial interval of 14.3 nm (Figure 1B). At the middle of myosin filaments, there are regions without myosin heads, which is called bare zone. Polarity of myosin heads extending from myosin filaments is reversed across the bare zone. Myosin S-2 rod serves as a hinge connecting myosin heads to myosin filament backbone, thus enabling myosin heads to swing away from myosin filaments. Meanwhile, actin filaments consist primarily of two helical strands of globular actin monomers (G-actin, MW 41,700) with a pitch of 35.5 nm (Figure 1C). Axial separation of actin monomers in actin filaments is 5.46 nm. In vertebrate skeletal muscle, actin filaments also contain tropomyosin and troponin.

In muscle, myosin and actin filaments are periodically arranged to form sarcomeres, which are structural and functional unit of muscle. As illustrated in Figure 1D, actin filaments extend from Z-line in either direction to penetrate in between myosin filament arrays, which are located central in each sarcomere. During muscle contraction, the length of each sarcomere shortens as a result of sliding between actin and myosin filaments, in such a way that actin filaments are further drawn in towards the center of myosin filaments, i.e. bare zone. The length of both myosin and actin filaments has been shown to remain constant during contraction and passive muscle stretch by a number of experiments, including (1) phase contrast microscopy of muscle fibers and myofibrils [1,2], electron microscopy of myofilaments [3], and X-ray diffraction of muscle fibers [4]. The fundamental question concerning the mechanism of muscle contraction is therefore, what makes myofilaments to slide past each other?

Muscle is regarded as a machine, converting chemical energy of ATP hydrolysis into mechanical work. i.e. force and motion resulting from myofilament sliding. Since both actin- and ATP-binding sites are located in myosin heads, myosin heads are regarded to play an essential role in muscle contraction. In 1969, H.E. Huxley [5] put forward a hypothesis, in which a myosin head first attach to the myosin-binding site on actin filament, undergoes a conformational change to produce unitary myofilament sliding, and then detach from actin filament (Figure 2). This hypothesis is supported by biochemical reaction steps on ATPase kinetics of actin and myosin in solution [6], in which myosin head (M) first attaches to actin (A) in the form of M•ADP•Pi, undergoes a conformational change associated with reaction, A•M•ADP•Pi → A•M+Pi+ADP, and then detaches from A on binding with next ATP to restore its original conformation associated with reaction, A•M+ATP → A+M•ATP → A+M•ADP•Pi. The first conformational change of myosin head producing myofilament sliding is called power stroke, while the subsequent restoration of original myosin head conformation is called

***Corresponding author:** Haruo Sugi, Department of Physiology, School of Medicine, Teikyo University, Tokyo, Japan, Tel/Fax: +81 484 78 4079; E-mail: sugi@kyf.biglobe.ne.jp

Received May 16, 2016; **Accepted** May 23, 2016; **Published** May 30, 2016

Citation: Sugi H, Chaen S (2016) Evidence against the Swinging Lever Arm Mechanism in Muscle Contraction Based on the Effect of Antibodies to Myosin Head. J Nanomed Nanotechnol 7: 377. doi:10.4172/2157-7439.1000377

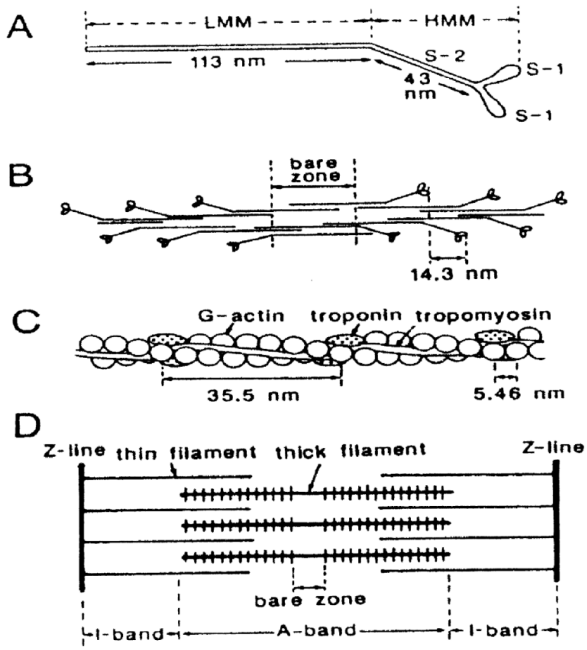

Figure 1: Structure of myosin and actin filaments and their arrangement in a sarcomere. (A) Diagram of myosin molecule. (B) Arrangement of myosin molecules in myosin filament. (C) Structure of actin filament. (D) Arrangement of myosin and actin filaments in a sarcomere. For further explanation, see text.

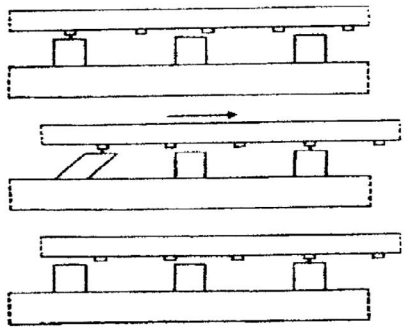

Figure 2: Diagram of attachment-detachment cycle between myosin heads extending from myosin filaments and corresponding myosin-binding sites on actin filaments. Periodicity of myosin head and that of myosin-binding sites differs from each other. Myosin head first attaches to actin filament (top), changes its configuration to move actin filament to the right (middle), and then detach from actin filament (bottom) [5].

recovery stroke. The cyclic reaction steps is therefore consistent with the attachment-detachment cycle showin in Figure 2, and are coupled with ATP hydrolysis (Figure 3). The central object of study in the research field of muscle contraction is therefore the molecular mechanism of myosin head power and recovery strokes coupled with ATP hydrolysis.

The Myosin Head Tilting Model

In 1971, Huxley and Simmons [7] presented a contraction model, which can be summarized as follows.

- Both myosin and actin filaments are assumed to be completely rigid.
- During muscle contraction, each myosin head, with its long axis perpendicular to actin filaments, first attaches to action filament, exerts a power stroke producing unitary myofilament sliding, and then detaches from actin filament.
- The conformational change of myosin head, producing power stroke, is the change in its angle of attachment from 90 to 45 degrees, i.e. tilting of the whole myosin head while attached to actin filament (Figure 4).
- The link connecting myosin heads to myosin filament backbone, i.e. myosin S-2, has a finite elasticity. If muscle length is held constant during contraction, so that myofilaments are not allowed to slide past each other, the tension in each myosin S-2 , which is stretched by the myosin head tilting, shows up as isometric tension in muscle (Figure 4B and 4C).

It has long been known that, when the length of an isometrically contracting muscle is quickly decreased by ~1% [8] (corresponding to a decrease in half sarcomere length of ~10 nm), the isometric tension falls to zero, and then starts redeveloping towards the initial value. The transient decrease in isometric tension following a ~1% release can be explained by the two component model [8], in which a muscle consists of elastic component (SEC) and contractile components CC) connected in series. In relaxed muscle, the SEC is slack and bears no tension (Figure 5A). When muscle contracts isometrically, the CC shortens internally to stretch the SEC, i.e. myosin S-2, so that tension generated by the stretched S-2 shows up as isometric tension. On application of a quick decrease in length (quick release) by ~1% to isometrically contracting muscle, the SEC is transiently made slack while myosin head restores its original position perpendicular to actin filament, thus producing in drop of isometric tension to zero. If the myosin S-2 is actually stretched by myosin head tilting as illustrated in Figure 4, a quick release applied to isometrically contracting muscle is expected to produce back-tilting of myosin heads, which should be detected as changes in equatorial X-ray diffraction pattern from contracting muscle. Contrary to this expectation, time-resolved X-ray diffraction studies failed to detect appreciable changes in equatorial X-ray diffraction pattern [9-12], indicating that the myosin head tilting model was not supported experimentally.

The Swinging Lever Arm Mechanism

As described above, the myosin head tilting model, which assumes movement of the whole myosin head, was not consistent with the X-ray

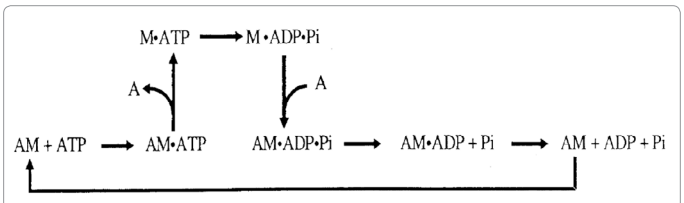

Figure 3: Diagram showing cyclic ATPase reaction steps [6].

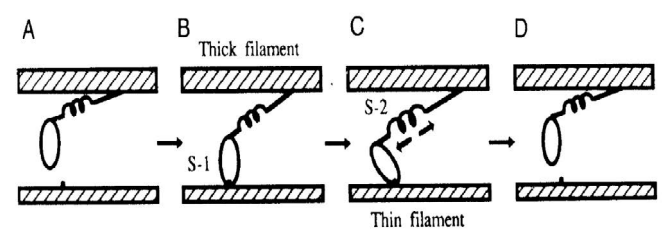

Figure 4: Myosin head tilting model. (A to D) Myosin S-1 head extending from myosin (thick) filament first attaches to actin (thin) filament, changes its angle of attachment to pull myosin S-2, and then detach from actin filament [7].

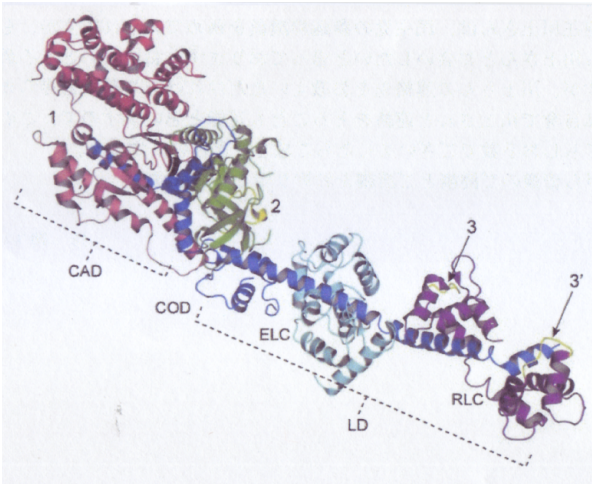

Figure 5: Atomic structure of myosin (S-1) head. Oval-shaped CAD consists of 25 kDa (green) and 50 kDa (red) and part of 20 kDa (dark blue) fragments of myosin heavy chain, while rod-shaped LD consists of the rest of 20 kDa fragment and two light chains (ELC, light blue and RLC, magenta). Approximate regions of attachment of anti-CAD, anti-RLR and anti-LD antibodies are indicated by numbers 1, 2 and 3 and 3', respectively).

diffraction experiments. Consequently, possible structural changes producing myosin head power stroke with amplitude of ~10 nm, is thought to be localized in a limited region within myosin head, so that myosin heads keep their angle of attachment to actin filament constant during the course of power stroke. To explore this possibility, it is essential to know atomic structure of myosin head in detail. Rayment and his coworkers [13-16] made pioneering studies on myosin head crystal, and succeeded in obtaining myosin head structure with high spatial resolution, shown in Figure 5. A myosin head consists of oval-shaped catalytic domain (CAD) and extended lever arm domain (LD, previously called neck region). Both actin-binding and ATP-binding sites are located in the CAD. The interface between the CAD and LD is called converter domain (COD). To obtain information about the molecular mechanism of myosin head power stroke, Rayment and his coworkers made extensive studies on nucleotide-dependent structural changes in crystals of truncated slime mold (*Dictyostelium*) myosin head, from which the LD was mostly detached except for its base remaining in the COD, because the truncated myosin head, without extended LD, easily formed crystals. Rather surprisingly, the crystal structure of the truncated slime mold myosin head was almost the same as that of vertebrate skeletal muscle myosin head, despite the two animals differ so far from each other. Their striking structural similarity might result from close packing of S-1 molecules when they form crystals, suggesting possible deformation of myosin head structures. Nevertheless, they studied nucleotide-dependent structural changes of truncated slime mold myosin head crystal, using a number of nucleotide analogs (14-16). It was found that, depending on the kind of nucleotide bound to myosin head, the remaining LD base in the COD, rotated by 60 degrees. This change in angle of LD base was taken to reflect pre- and post-power stroke states of myosin head. It seems, however, not clear whether the change in angle of LD base can actually produce rotation of the whole LD, producing myosin head power stroke, if a large torque necessary to swing the whole LD against large external loads is taken into consideration. Smith and Rayment [17] have reached an idea that myosin head can take two different states, i.e. open and closed states, depending on the kind of nucleotide bound to myosin head. After a number of considerations regarding possible localized structural changes in and around the COD, coupled with ATP hydrolysis, the swinging lever arm hypothesis was constructed. In this hypothesis, myosin head power stroke is caused by active rotation of the LD around the COD, while myosin head CAD remains rigid, and moves by ~10 nm during power stroke without changing its angle of attachment to actin filaments [18], as illustrated in Figure 6. The swinging lever arm hypothesis is now cited in many textbooks, constituting a dogma on the mechanism of muscle contraction without concrete experimental support.

Absence of Inhibitory Effect of Anti-RLR Antibody on Muscle Contraction

Sutoh et al. prepared two different monoclonal antibodies (IgG) directed to various regions within myosin head, and successfully made molecular mapping of myosin head [19]. One antibody directed to junctional peptide between 50-KDa and 20 kDa segments of myosin heavy chain in the CAD (anti-CAD antibody), while the other antibody directed to reactive lysine residue (Lys 83) located close to the CAD-LD interface, i.e. the COD (anti-RLR antibody). In addition to the above two antibodies, Sugi and his coworkers prepared another antibody directed to peptides in two light chains in the LD (anti-LD antibody) [20]. These antibodies diffuse into skinned muscle fibers to bind with myosin heads with a fairly high affinity [20,21]. Approximate points of attachment of the three antibodies in myosin head are indicated by numbers 1, 2 and 3, 3' in Figures 5 and 6. Unexpectedly, Sugi et al. [21] found that Anti-RLR antiby had no appreciable effect on the force-velocity relation of Ca^{2+}-activated chemically skinned muscle fibers even in high concentrations (up to 2 mg/ml). As illustrated in Figure 7, the force-velocity relation did not change appreciably before and after administration of anti-RLR antibody. This indicates that anti-RLR antibody does not affect both isometric force development and the maximum unloaded shortening velocity in Ca^{2+}-activated muscle fibers. In contrast, anti-COD antibody completely eliminated in vitro actin-myosin sliding in low concentrations, but had no appreciable effect on actin-activated S-1 ATPase activity [21], indicating that in vitro actin-myosin sliding is not necessarily a good model for muscle contraction. As IgG molecule is nearly the same in size as myosin head, its binding to reactive lysine residue in the COD is expected to greatly inhibit

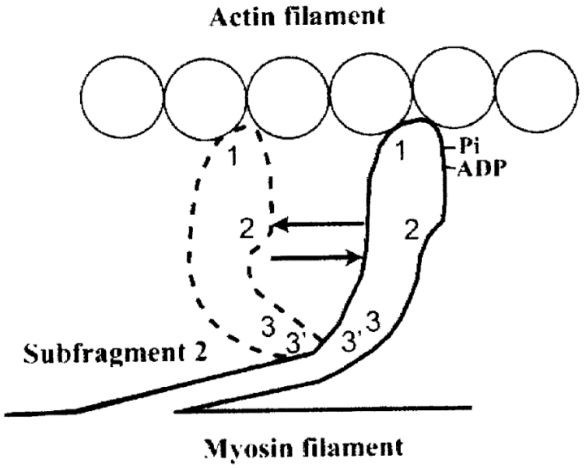

Figure 6: Conformational change of myosin (S-1) head before (solid line) and after (broken line) power stroke. Numbers indicate approximate regions of attachment of anti-CAD (1) , anti-RLR (2), and anti-LD (3 and 3') antibodies. Note that angle of attachment of CAD to actin filament remains unchanged.

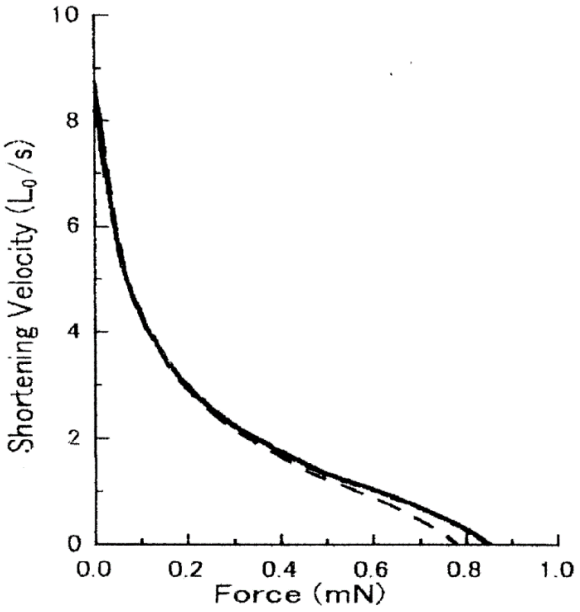

Figure 7: Force-velocity relation of Ca^{2+}-activated single muscle fiber before (solid line) and after (broken line) application of anti-RLR antibody (2 mg/ml) [21].

local structural changes in and around the COD, which is assumed to produce active rotation of the LD around the COD. Contrary to this expectation, however, anti-RLR antibody had no appreciable effect on Ca^{2+}-activated muscle fiber contraction (Figure 7), strongly suggesting that the lever arm mechanism may not be responsible for myosin head power stroke.

In contrast with the finding of Sugi et al. that anti-RLR antibody shows no appreciable effect on muscle contraction [21], Muhlad et al. reported that trinitrophenylation of reactive lysine residue inhibits both ATP-dependent in vitro actin-myosin sliding and actin-activation of myosin MgATPase activity [22]. They interpret inhibition of actin-myosin sliding and actin-activation of myosin ATPase activity as being due to mechanical crush of structures in and around the COD, so that the local structural changes were impaired. The apparent discrepancy in the results between the two research groups may be accounted for in the following way; chemical modification of reactive lysine residue inevitably changes three-dimensional (3-D) structures not only in and around the COD, but structures in and around ATP-binding site in myosin head CAD, to produce inhibition in both myosin head power stroke and actin-activation of myosin ATPase activity. Meanwhile, binding of anti-RLR antibody to reactive lysine residue may not alter 3-D structures in and around the COD.

ATP-Induced Myosin Head Power and Recovery Strokes Can Take place in Myosin heads Position-Marked with Antibodies

Sugi and his coworkers developed a novel method, in which dynamic structural changes of hydrated myosin filaments in response to ATP can be visualized and recorded, using the gas environmental chamber (EC) attached to an electron microscope [23-25]. To record ATP-induced myosin head movement, individual myosin heads were position-marked with gold particles (diameter, 20 nm) via anti-CAD, anti-RLR or anti-LD antibodies (Figure 8). In response to applied ATP, individual myosin heads were found to move away from myosin filament bare zone, across which myosin head polarity was reversed (Figure 9). Since the experimental system does not contain actin filaments, the observed myosin head movement is regarded as myosin head recovery stroke associated with reaction, M+ATP → M•ATP → M•ADP•Pi. The average amplitude of ATP-induced myosin head movement, mostly regarded to take place under practically zero external load, was 6.14 ± 0.09 nm (mean ± s.e.m, n=1962) at the distal end of CAD, 6.14 ± 0.22 nm (n=1112) at the COD where RLR is located, i.e. at the proximal end

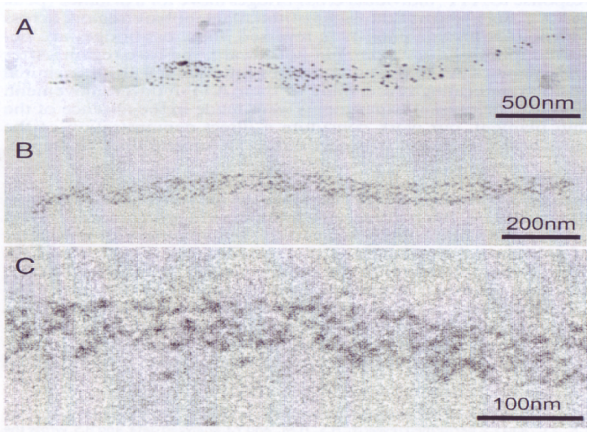

Figure 8: Electron micrographs of synthetic myosin filaments with a number of gold particles attached to individual myosin heads via antibodies to myosin head. (A,B) The whole spindle-shaped myosin filaments with tapered ends. (C) Enlarged view of myosin filament shown in A [24].

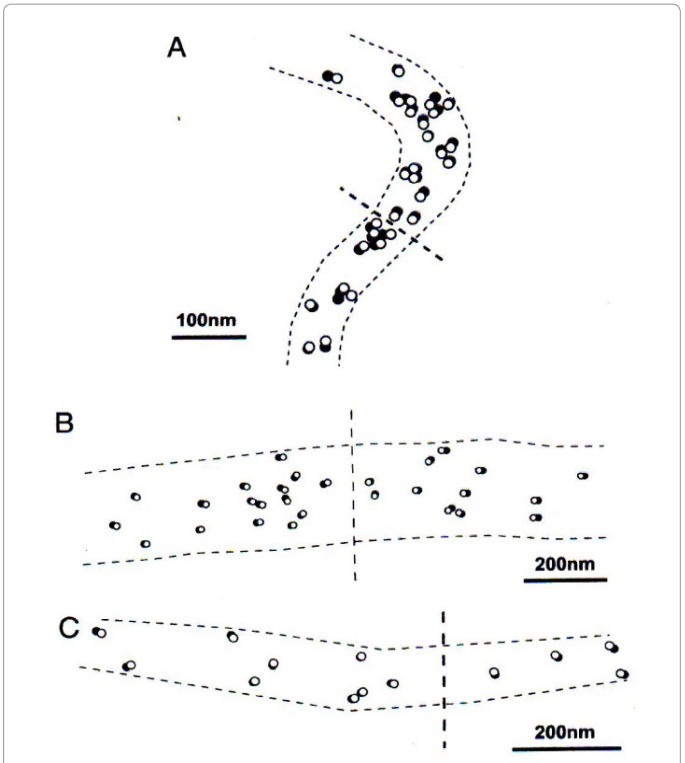

Figure 9: Examples of records showing ATP-induced myosin head movement at both sides of myosin filament bare zone (broken line), across which myosin head polarity is reversed. Open and filled circles (diameter, 20 nm) are drawn around center of mass position of gold particles before and after ATP application, respectively. Note that myosin heads move away from bare zone [24].

of CAD, and 3.55 ± 0.11 nm (n=981) at the LD. These results well agree with the mode of myosin head power stroke, in which myosin head CAD remains rigid and does not change its attachment angle with actin filament, as predicted by the swinging lever arm hypothesis (Figure 6), but do not support the lever arm mechanism, since attachment of bulky anti-RLR antibody to the COD is expected to greatly inhibit structural changes causing active rotation of the LD around the COD.

More recently, Sugi et al. succeeded in recording ATP-induced power stroke in individual myosin heads in hydrated mixture of actin and myosin filaments (Figure 10) [26]. Before ATP application, myosin heads extending from myosin filaments form rigor linkages with actin filaments. On ATP application, myosin heads detach from actin filaments to perform power stroke. As the rate of ATP release from ATP-containing microelectrode is limited, the ATP concentration around myosin heads is <10 μM [23]. This situation resembles that of optical trap experiments, in which a single myosin head fixed on a bead surface is brought into contact with an actin filament; before ATP application, myosin head form rigor linkage with actin filaments. On binding with ATP, it detaches from actin filament and start repeating power and recovery strokes. At μmolar ATP concentrations, myosin head has to wait for next ATP for a considerable time, so that, under a high trap stiffness, myosin heads exhibit force transients up to >1s duration each being separated by up to >1 s. This is the reason why Sugi et al. [26] succeeded in recording ATP-induced myosin head power stroke despite limited time resolution of ~1s. Considering the myosin head concentration in synthetic myosin filaments and ATP concentration around them, only a small proportion of myosin heads can be activated by ATP to perform power stroke, while the majority of myosin heads continue forming rigor linkages with actin filaments. In such a condition, ATP-activated myosin heads can not produce gross myofilament sliding, and only stretch adjacent elastic structures during their power stroke. This condition is similar to that in muscle fibers with two ends fixed in position, i.e. nominally isometric condition.

In this nominally (or nearly) isometric condition, the average amplitude of ATP-induced myosin head power stroke was 3.3 ± 0.2 nm (mean ± SD, n=732) at the distal region of the CAD (myosin heads, position-marked with anti-CAD antibody) and 2.5 ± 0.1 nm (n=613) at the proximal region of the CAD (myosin heads, position-marked with anti-RLR antibody), indicating that the power stroke amplitude is smaller at the proximal region than at the distal region of the CAD (*t*-test, $p<0.01$). If ionic strength of experimental solution was reduced by reducing KCl concentration from 120 to 20 mM (corresponding to a reduction of ionic strength μ from 170 to 50 mM), the average amplitude of ATP-induced power stroke was found to increase significantly to 4.4 ± 0.1 nm (mean ± SD, n=361) at the distal region of the CAD, and to 4.3 ± 0.2 nm (n=305) at the proximal region of the CAD. The amplitude of myosin head power stroke was not statistically different between the distal and the proximal regions of the CAD. The increase in amplitude of myosin head power stroke at low ionic strength is consistent with our previous report [27] that the maximum isometric force in Ca^{2+}-activated muscle fibers increases ~twofold at low ionic strength; if force generated by individual myosin heads also increase ~twofold in the filament mixture mounted in the EC, they can stretch adjacent elastic structures more markedly by their power sroke.. The results obtained on the amplitude of myosin head power stroke in the standard and low ionic strengths are summarized diagrammatically in Figure 11, in which myosin head CAD is assumed to be rigid. It can be seen that myosin head CAD does not keep its angle of attachment to actin filament constant at 90 degrees if external load on it is too large (Figure 11B). On the other hand, if external load on it is not so large, myosin head keeps the attachment angle at 90 degrees during power stroke (Figure 11C). Together with the result that, myosin head CAD keeps the same configuration when they perform strokes freely without external load [20], we have an impression that, under moderate external loads, globular-shaped myosin head CAD has a property to move without tilting motion irrespective of whether actin filaments are present or absent.

Essential Role of Myosin LD and Subfragment-2 Overlooked in the Swinging Lever Arm Hypothesis

Sugi et al. also found that anti-LD antibody inhibits development of Ca^{2+}-activated isometric force development in Ca^{2+}-activated muscle fibers in a dose-dependent manner [21]. As can be seen in Figure 12, force-velocity curves obtained in the absence and in the presence of anti-LD antibody were identical if they are scaled with respect to the maximum isometric force, indicating that anti-LD antibody does not affect the maximum unloaded shortening velocity as well as the shape of force-velocity curves. This implies that the reduction of isometric force by anti-LD antibody results from decrease in the number of myosin heads involved in isometric force generation; in other words, myosin heads to which anti-LD antibody binds at their LD region can no longer perform power stroke producing force and motion in muscle. Simultaneous recordings of Ca^{2+}-activated isometric force development and MgATPase activity indicated that anti-LD antibody had no effect on MgATPase activity (Figure 13) despite its effect in

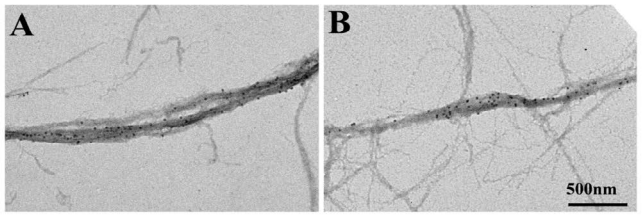

Figure 10: Conventional low magnification electron micrographs of actin and myosin filament mixture. Thick myosin filaments, with gold particles attached to myosin heads, are surrounded by thin actin filaments [26].

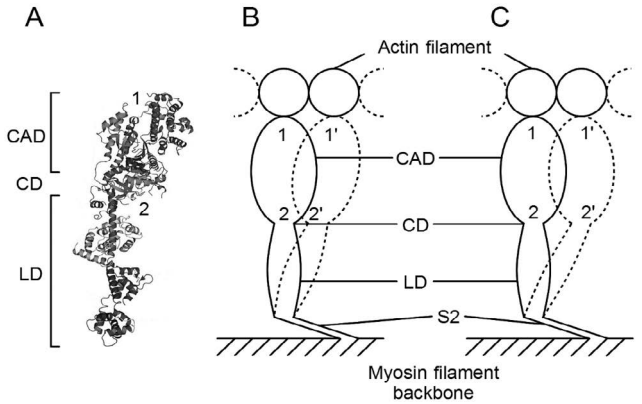

Figure 11: Change in the mode of myosin head power stroke depending on experimental conditions. (A) Diagram of myosin head, consisting of CAD, COD and LD and connected to myosin filament backbone. Approximate regions of attachment of anti-CAD and anti-RLR antibodies are indicated by numbers 1 and 2, respectively. (B) The mode of myosin head power stroke in nominally isometric condition at standard ionic strength. The amplitude of movement is larger in CAD region than in COD region. (C) The mode of myosin head power stroke at low ionic strength. The amplitude of movement is similar at both CAD and COD regions [26].

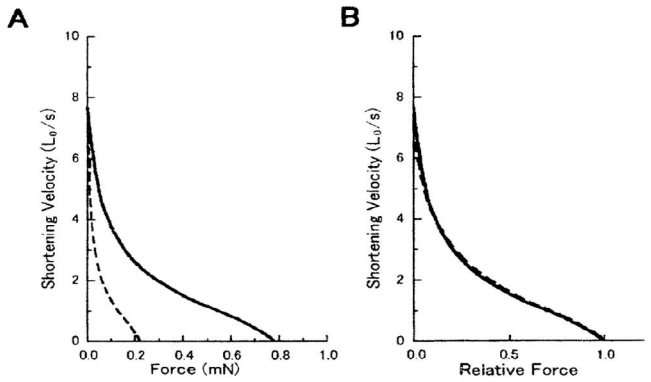

Figure 12: Effect of Anti-LD antibody on the force-velocity curves in Ca^{2+}-activated single skinned muscle fibers. (A) Force-velocity curves before (solid line) and after administration (broken line) of anti-LD antibody (1.5 mg/ml). (B) The same force-velocity curves with forces normalized relative to maximum value [21].

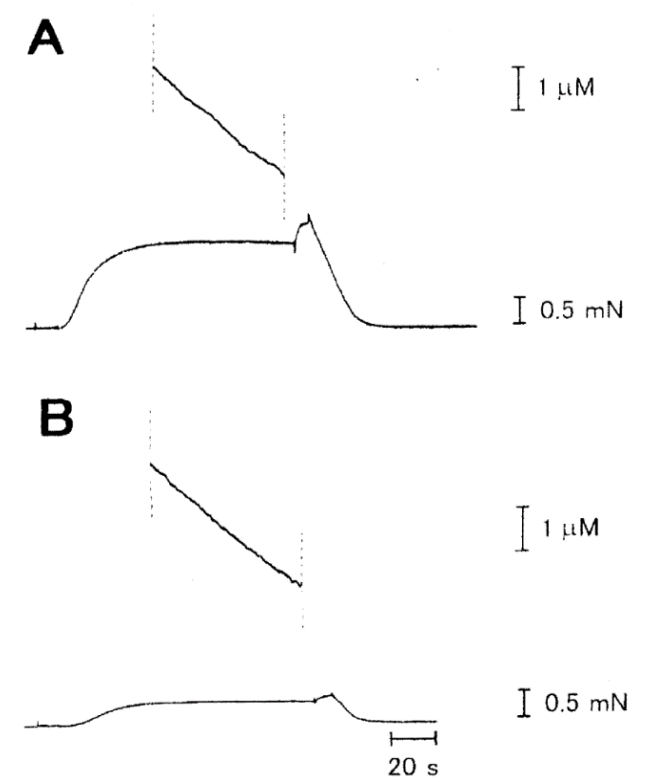

Figure 13: Simultaneous recordings of MgATPase (upper traces) and isometric force (lower traces) in Ca^{2+}-activated skinned muscle fibers. Records A and B were taken before and at 15 min after application of anti-LD antibody (2 mg/ml). Muscle fiber ATPase activity was measured by measuring NADH fluorescence. Note that MgATPase activity, as measured by the slope of upper traces, does not change appreciable, despite marked decrease in isometric force [21].

reducing isometric force development. Polyclonal antibody to myosin S-2 (anti-S-2 antibody) kindly provided from the late Professor Harrington to us, also found to produce reduction and eventual elimination of isometric force development in Ca^{2+}-activated muscle fibers without affecting MgATPase activity (Figure 14) [28]. As with anti-LD antibody, anti-S-2 antibody had no appreciable effect on the maximum unloaded shortening velocity in Ca^{2+}-activated muscle fibers [28]. From these results, it seems clear that both myosin head LD and myosin S-2 play an essential role in muscle contraction. The ineffectiveness of the two antibodies on MgATP ase activity of muscle fibers may result from that ATP-binding site in myosin head CAD is geographically distant from the LD and the S-2.

Conclusion

In this article, we presented evidence against the swinging lever arm mechanism of muscle contraction, constituting a new dogma in the research field of muscle contraction. This mechanism is, however, constructed from studies on nucleotide-dependent structural changes in crystals of truncated myosin head, from which both LD and myosin

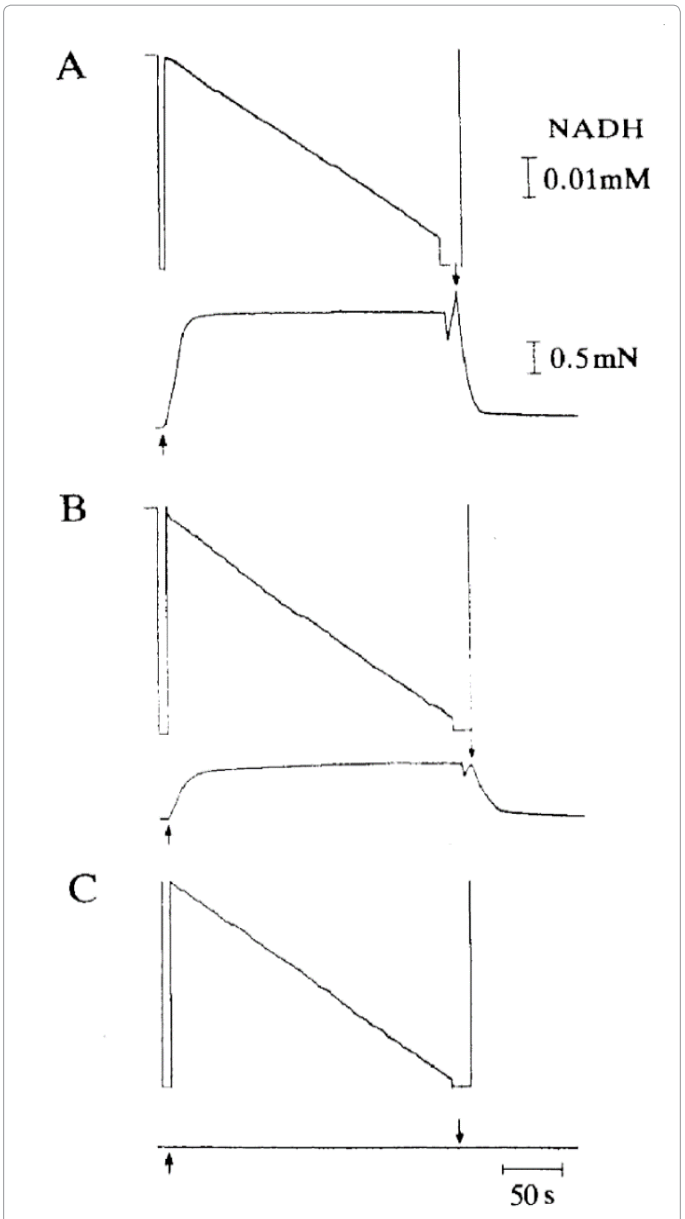

Figure 14: Simultaneous recordings of MgATPase activity (upper traces) and isometric force (lower traces) in Ca^{2+}-activated skinned muscle fibers, before (A), and 100 min (B) and 150 min (C) after application of anti-S-2 antibody, prepared by Harrington. Note that isometric tension is gradually reduced to zero with time after application of anti-S-2 antibody, while MgATPase activity does not change appreciably [28].

S-2 are removed, with an assumption that the observed structural change in truncated myosin head also holds in whole myosin head. The idea of active rotation of the LD around the COD seems to be non-realistic, if a large torque required to swing lever arm domain over a large distance (~10 nm) is taken into consideration. As a matter of fact, Ca^{2+}-activated muscle contraction is not inhibited by binding of bulky anti-RLR antibody to the COD, indicating that the lever arm mechanism is unlikely. It should also be noted that ATP-dependent in vitro actin-myosin sliding is not necessarily a good model for muscle contraction taking place in the 3-D myofilament-lattice. On the other hand, as we have shown using anti-LD and anti-S-2 antibodies, myosin head LD and myosin S-2 play an essential role in muscle contraction. Much more attention should be focused on the LD and the S-2. Muscle is still filled with a number of unsolved mysteries to be investigated in future.

Acknowledgements

We wish to thank Professor Masaru Tanokura and Takuya Miyakawa for providing us antibodies to myosin head. Our thanks are also due to Professor Takakazu Kobayashi and Drs. Hiroki Minoda and Tsuyoshi Akimoto for their cooperation to carry out experiments.

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