Biomedical Discoveries

Review Article

Shortcomings of the Swinging Lever Arm Hypothesis as Revealed by the Effect of Antibodies to Myosin Molecule

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Abstract

Background: The swinging lever arm (SLA) hypothesis of muscle contraction has been constructed from nucleotide-dependent structural changes in crystals of truncated myosin head, from which lever arm domain (LD) is removed except for its base. Using inorganic compounds as nucleotide analogs, the LD base was found to rotate by ~60° in a nucleotide-dependent manner. This led to the proposal that myosin head power stroke is caused by active rotation of myosin head catalytic domain (CAD) around converter domain (COD).

Shortcomings of the SLA hypothesis: The SLA hypothesis contain uncertainties arising from the use of slime mold myosin, the use of inorganic compounds as nucleotide analogs, and the bold assumption that the whole LD also rotates by 60°. In addition, people concerned with the construction of SLA hypothesis completely ignore published papers indicating important role of myosin subfragment-2 (S-2).

Evidence against the SLA hypothesis: The following results constitute evidence against the SLA hypothesis: (1) Both antibody to myosin head LD and that to myosin S-2 inhibit muscle contraction without changing MgATPase activity; (2) Antibody to myosin head COD has no effect on muscle contraction, and also on ATP-induced myosin head power and recovery strokes. These results also demonstrate functional communication between myosin S-2 and distal region of myosin head CAD where actin-myosin interaction takes place. (3) Experiments using the gas environmental chamber show that myosin head CAD does not necessarily move parallel to actin filaments, contrary to prediction of the swinging lever arm hypothesis.

Conclusion: Muscle contraction mechanism should be reconsidered in future, taking the essential role of myosin head LD and myosin S-2 into consideration.

Introduction

A myosin head consists of oval-shaped catalytic domain (CAD), where both actin- and ATP-binding sites are located, small converter domain (COD), and rod-shaped lever arm domain (LD), which is further connected to myosin filament backbone via myosin subfragment -2 (S-2, not shown) (Figure 1). In relaxed muscle, myosin head (M) is believed to be in the form of M-ADP-Pi. In contracting muscle, M-ADP-Pi binds with actin filament (A) to perform power stroke producing unitary myofilament sliding, associated with reaction M-ADP-Pi + A → A-M-ADP-Pi → A-M + ADP + Pi. M detaches from A upon binding with next ATP, and performs recovery stroke, associated with reaction A-M + ATP → A + M-ATP → A + M-ADP-Pi, to repeat cyclic alternate power and recovery strokes [1] (Figure 2). For further explanations, see text.
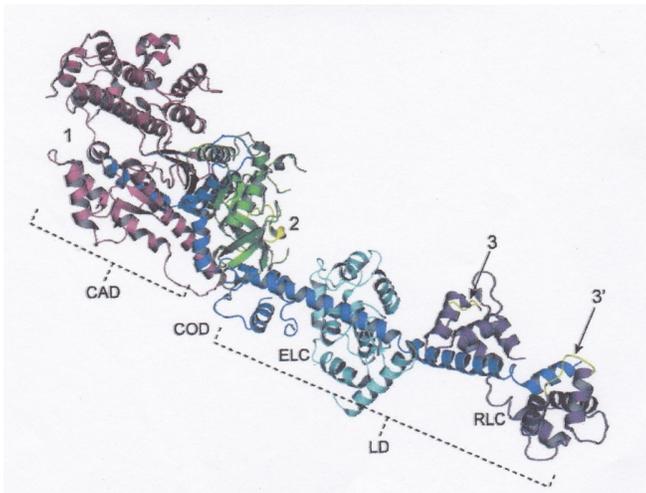
Figure 1: Myosin head structure showing approximate regions of attachment of antibodies 1(anti-CAD antibody), 2 (anti-COD antibody) and 3(anti-LD antibody) by numbers 1,2 and 3 and 3' respectively [18,20]. Catalytic domain (CAD) comprises 25K (green), 50K (red) and part of 20K (dark blue) fragments of myosin heavy chain, while lever arm domain (LD) comprises the rest of 20K fragment and essential (ELC) and regulatory (RLC) light chains. CAD and LD are connected via small converter domain (COD). Location of peptides around Lys 83 and that of two peptides (Met 58 ~ Ala 70 and Lue 106 ~ Phe 120) in LD are colored yellow. From [18].
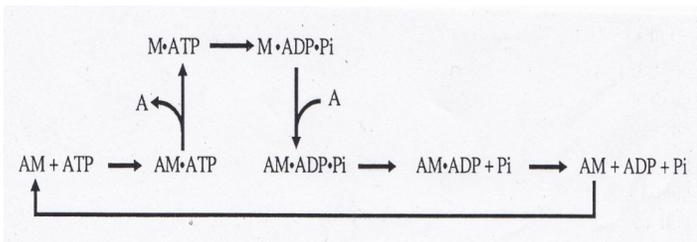

Figure 2: Lymn-Taylor Scheme of cyclic actomyosin ATPase reaction steps [1].

The conformational changes producing power and recovery strokes were first regarded as being due to tilting of myosin head CAD [2], but this mechanism was denied mainly by time-resolved X-ray diffraction studies that myosin head CAD does not change its angle to actin filament [3-5]. At present, myosin head CAD is believed to keep its attachment angle at 90° during the course of power and recovery strokes, as illustrated in Figure 3. As can be seen in the figure, myosin head CAD is assumed to be rigid, and its movement parallel to actin and myosin filaments should be accompanied by change in angle around the CAD-LD junction (i.e. COD) and also that around the LD-S-2 junction. Up to the present time, there is no definite experimental evidence as to which one or ones of the two junctions is responsible for myosin head power and recovery strokes.
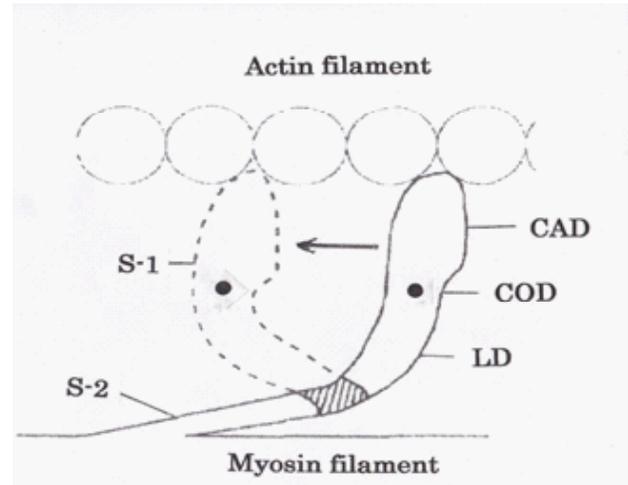

Figure 3: Diagram showing myosin head power stroke (arrow). Myosin head CAD, COD, LD, and myosin S-2 are shown by characters CAD, COD, LD, and S-2, respectively. Filled circles shows approximate pivot point, around which CAD and LD rotate actively around COD. Note that myosin head CAD keeps its angle of attachment to actin filament at 90°. Shaded area indicates LD-S-2 boundary, where change in LD-S-2 angle is regarded as passive in the swinging lever arm hypothesis.

Construction of the Swinging Lever Arm Hypothesis Based on Crystallographic studies: Its Shortcomings

Crystallographic studies have been made intensively using crystals of truncated slime mold myosin head, from which the LD region was removed except for its base remaining at the end of the truncated myosin. By removing the LD, oval-shaped truncated myosin molecules formed crystals, which were found to be almost the same as those prepared from rabbit skeletal muscle myosin [6,7]. The close similarity between rabbit skeletal and slime mold myosins is surprising if the extreme difference between the two materials (one, vertebrate animal in the Animal Kingdom, while the other primitive organism in the Plant Kingdom) is taken into consideration. It seems possible that, due to close packing of molecules during crystal formation, important differences in crystal structure, especially regions related to their physiological function, might be masked and overlooked.

Nevertheless, the investigators intended to obtain information about nucleotide-dependent structural changes of truncated slime mold myosin head crystals, by using inorganic compounds instead of ATP and ADP. The nucleotide-dependent structural change that they found was rotation of the LD base remaining at the end of truncated myosin by ~60° [6-10]. Although I appreciate their efforts in obtaining interesting results, their process in constructing the detailed mechanism of structural changes around the ATP binding site accompanying ATP hydrolysis, which produces active rotation

of myosin head CAD and LD around COD, i.e. the swinging lever arm mechanism, there are serious shortcomings in constructing a general mechanism of muscle contraction, which can be listed as follows:

(1) The validity to consider that the structure of slime mold myosin crystal is identical with that of rabbit skeletal muscle myosin is not proved.

(2) The validity of using inorganic compounds as analogs of ATP and ATP is not proved.

(3) The validity of assuming the active rotation of massive myosin head CAD around small COD is not certain. The mechanism reminds us of an idiom, "the tail waggles the dog". It seems rather implausible that structures confined to the small COD region can generate torque sufficient to move massive CAD, which bears large load generated by myofilament sliding.

(4) The last and the greatest shortcoming in constructing swinging lever arm hypothesis is complete ignorance of references, reporting essential role of myosin S-2 and myosin head LD in producing muscle contraction.

In this article, we will discuss the last issue by describing experimental results obtained by Harrington and his coworkers and by us.

Evidence for Close Communication between Myosin S-2 and Myosin Head CAD

William F. Harrington was a prominent polymer scientist, and got interested in muscle contraction mechanism and focused attention to myosin S-2 hinge region, with which myosin heads can move towards actin filament. He used the enzyme probe method, with which structural changes of the hinge region during contraction was detected by comparing the rate of chymotrypsin splitting of the hinge region in relaxed, contracting and rigor muscle fibers. It was found that the hinge region was split rapidly by chymotrypsin in contracting fibers, but not readily split in relaxed and rigor fibers [11] (Figure 4). Based on this and other experiments, Harrington proposed a contraction model, in which helix-coil transition occurring in S-2 hinge region contributes to muscle force generation [12]. To prove the validity of his helix-coil transition mechanism in muscle contraction, Harrington prepared a polyclonal antibody to myosin S-2 region (anti-S-2 antibody), and showed that the antibody reduced isometric force development of skinned fibers, and slowed down myofibrillar shortning [13,14] In 1992, (Figure 5) Harrington wrote to me,"Muscle investigators tend to ignore my hypothesis. Please work with us on the effect of anti-S-2 antibody on muscle contraction, using the antibody prepared in my laboratory". I agreed with him with pleasure, and examined the effect of anti-S-2 antibody on muscle contraction characteristics with the following results. At that time, Harrington

seemed to emphasize close communication between S-2 and myosin head CAD during contraction, rather than to insist helix-coil transition in S-2.
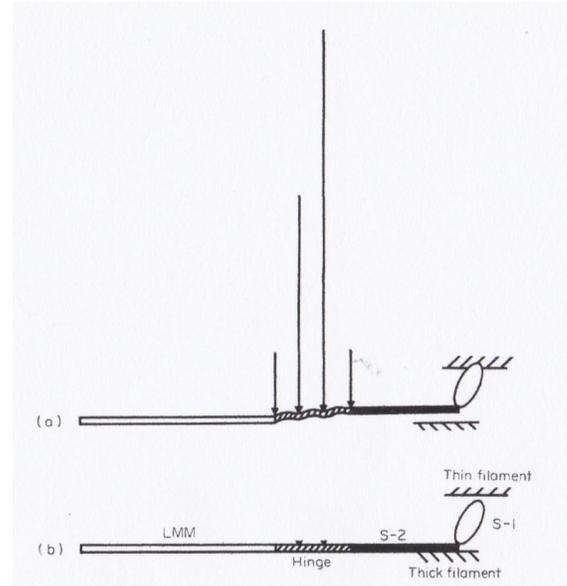

Figure 4: Diagrams illustrating chymotryptic cleavage of myosin S-2 region in Ca^{2+}-activated (a) and in relaxed and rigor fibers (b). Normalized cleavage rate constants are indicated by length of vertical arrows [12].
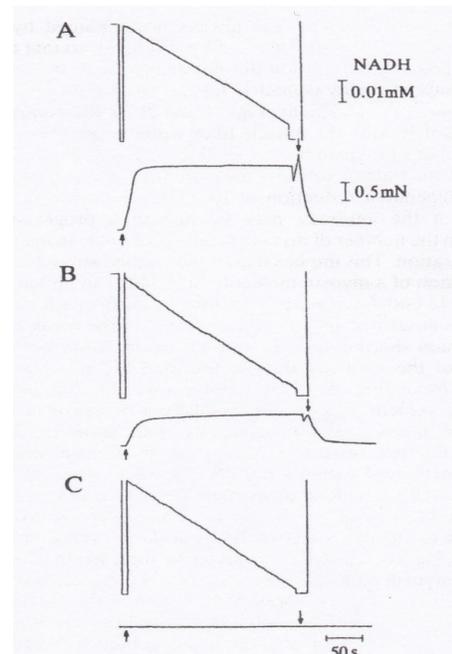

Figure 5: Simultaneous recordings of MgATPase activity (upper traces) and Ca^{2+}-activated isometric force development (lower traces) in skinned muscle fibers before (A), and 100min (B) and 150min (C) after application of anti-S-2 antibody (1.5mg/ml). Note that the slope of ATPase records

(decrease in NADH), representing MgATPase activity, remains unchanged even when isometric force is reduced to zero. Times of application of contracting and relaxing solutions are indicated by upward and downward arrows, respectively [15].

As shown in Figure 5, anti-S-2 antibody reduced Ca^{2+}-activated force development in a time-dependent manner, while MgATPase of muscle fiber remained unchanged even when isometric force was reduced to zero [15]. Muscle fiber stiffness, measured by applying small sinusoidal length changes, decreased in parallel with isometric force, indicating that the reduction of isometric force is accompanied by decrease in the number of myosin heads generating isometric force (Figure 6) [15]. Despite the decrease in the number of myosin heads, the maximum shortening velocity Vmax of the fiber, determined by applying ramp decreases in force, did not change appreciably (Figure 7) [15]. This unexpected results indicate that myosin heads, with anti-S-2 antibody attached to myosin, no longer participate in muscle force generation, but do not contribute to internal resistance against fiber shortning. This implies that anti-S-2 antibody can influence actin-myosin binding at the distal region of myosin head CAD by attaching to S-2 region. When I informed Dr. Harrington of our results, he was extremely happy and stated "Your results clearly demonstrated the close communication between S-2 and CAD regions. It is indeed a triumph!". Very sadly, however, Dr. Harrington died suddenly immediately after publication of our paper describing the above results. On the occasion of his memorial conference held in Johns Hopkins University, I was asked by Mrs. Harrington to be the first speaker, since she well knew that her husband died happily, being satisfied with our results.
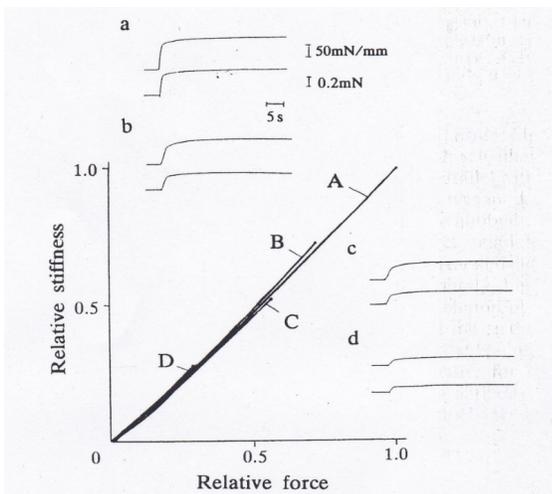

Figure 7: Effect of anti-S-2 antibody on force-velocity curves of Ca^{2+}-activated skinned muscle fibers, obtained at various levels of steady isometric force. (A) Force-velocity curves obtained before (control) and at 30, 60 and 90min after application of antibody (1.5mg/ml). Note that the maximum shortning velocity Vmax remains constant despite marked reduction of isometric force. (B) The same force-velocity curves as in (A), normalized with respect to peak steady isometric forces. Note that the curves are identical [15].

Figure 6: Relation between muscle fiber stiffness and Ca^{2+}activated isometric force in the presence of anti-S-2 antibody (1.5mg/ml). Stiffness versus force corves A, B. and C were obtained before and at 30, 60, and 90min after application of antibody, respectively (Insets). [15].
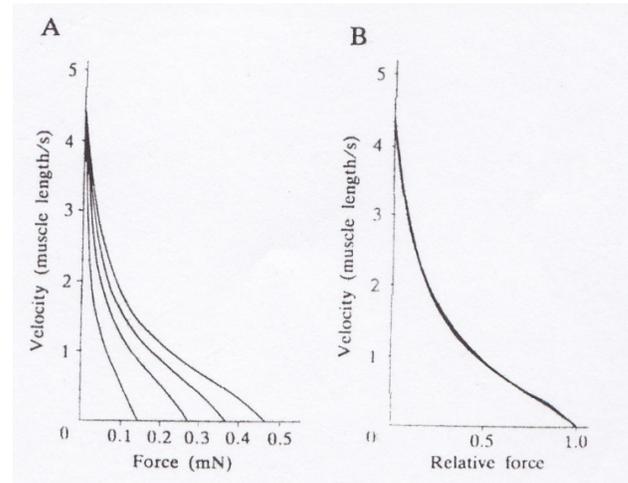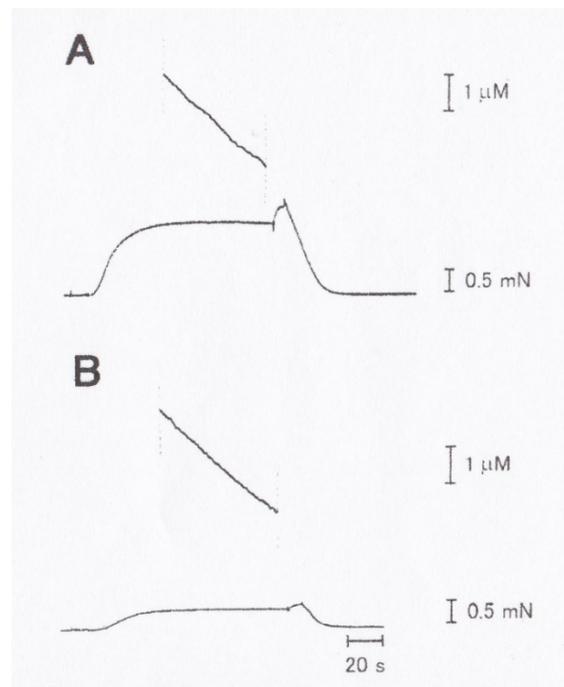

Figure 8: Simultaneous recordings of MgATPase activity (upper traces) and Ca^{2+}-activated isometric force development (lower traces) in skinned muscle fibers before (control) (A) and after application of anti-LD antibody (2.0mg/ml). Note that the slope of upper traces, representing MgATPase activity remains unchanged despite marked reduction of isometric force [16].

Although Harrington's sudden death prevented us from further discussing implications of the results, I was aware the importance of the result that, anti-S-2 antibody had no appreciable effect on the MgATPase activity of the fiber, even when isometric force was reduced to zero (Figure 5). In the Lymn-Taylor scheme [1], it is generally believed that the strength of actin-myosin (A-M) binding is weak in A-M-ADP-Pi, and strong in A-M-ADP and A-M; the weak to strong transition of A-M binding is associated with reaction, A-M-ADP-Pi → A-M-ADP + Pi. Consequently, the attachment-detachment cycle between A and M should be coupled with the weak-to-strong transition of A-M binding. Contrary to this concept, however, it is found that, muscle fibers, in which Ca^{2+}-activated force generation is completely eliminated by anti-S-2 antibody, still hydrolyze ATP at a rate equal to the control (Figure 5). This implies that myosin heads, with anti-S-2 antibody attached, still hydrolyze ATP at a normal rate without formation of strong A-M binding, since such myosin heads are readily detached from actin, they do not contribute muscle fiber stiffness in response to small sinusoidal length changes, and also do not contribute to internal resistance against fiber shortning. Similar results have been obtained from muscle fibers with anti-LD antibodies prepared by us. As can be seen in Figure 8, MgATPase activity does not change appreciably while Ca^{2+}-activated force is markedly reduced [16]. Recording of force-velocity curves also indicated that, despite the marked reduction of Ca^{2+}-activated isometric force, the maximum velocity of shortening did not change appreciably in anti-LD treated fibers [16]. These findings indicate that there is a communication pathway, i.e., myosin (S-2) → myosin head LD → distal region of myosin head CAD, where myosin head binds with actin. At present, we have no definite idea about the underlying mechanism of the S-2 to CAD communication. It seems possible, however, that the transmission of communication is made by simple mechanical strain along the alpha-helical structures in S-2 and LD, and this mechanism of transmission of communication may not work if antibodies are attached to myosin S-2 or myosin head LD.

Evidence against the Essential Role of Myosin Head COD in the Swinging Lever Arm Mechanism

When we succeeded in recording ATP-induced myosin head power and recovery strokes in hydrated rabbit skeletal muscle myosin filaments using the gas environmental chamber [17-19], we position-marked individual myosin heads by attaching gold particles via antibody to myosin head CAD (anti-CAD antibody) and that to myosin head COD (anti-COD antibody) [20], and regardless of which of the two antibodies was used, we always observed definite myosin head movements coupled with ATP hydrolysis. On the contrary, Muhlrad et al. [21] report that chemical modification (trimethylphenylation) of reactive lysine residue in myosin head COD inhibit both MgATPase activity and contraction of muscle fibers. The results have been explained as being due to collision of structures taking place in COD as the result of chemical modification of lysine residue, thus supporting the essential role of the COD serving as the pivot for active rotation of CAD and LD around COD.

The discrepancy between Muhlrad's results and our electron microscopic results stated above may be due to that chemical modification of lysine residue may produce irreversible structural changes not only in COD, but also in CAD, to result in inhibition of both MgATPase activity and contraction. On the other hand, the attachment of antibodies to their epitopes is mild and reversible. The ineffectiveness of the attachment of massive antibody molecules to myosin head COD, (Figure 9) seems to preclude the mechanism of active rotation of CAD and LD around COD.

In addition, our electron microscopic recording of myosin head power stroke in hydrated actin-myosin filament mixture [19] also serves as evidence against the swinging lever arm hypothesis. The proportion of myosin heads to be activated with applied ATP is very small due to limited amount of iontophoretically applied ATP, and as the result, activated myosin heads only stretch adjacent elastic structures without producing gross myofilament sliding (i.e. nearly isometric condition). As shown in Figure 10, the amplitude of power stroke is significantly larger at the distal region of CAD than at COD (B) in the standard ionic strength. Meanwhile, at low ionic strength which enhances Ca^{2+}-activated isometric force ~twofold [22], the amplitude of power stroke increases appreciably at both CAD and COD, so that the power stroke amplitude becomes equal to each other (C). These results clearly indicate that myosin head power stroke does not necessarily obey predictions of the swinging lever arm hypothesis.
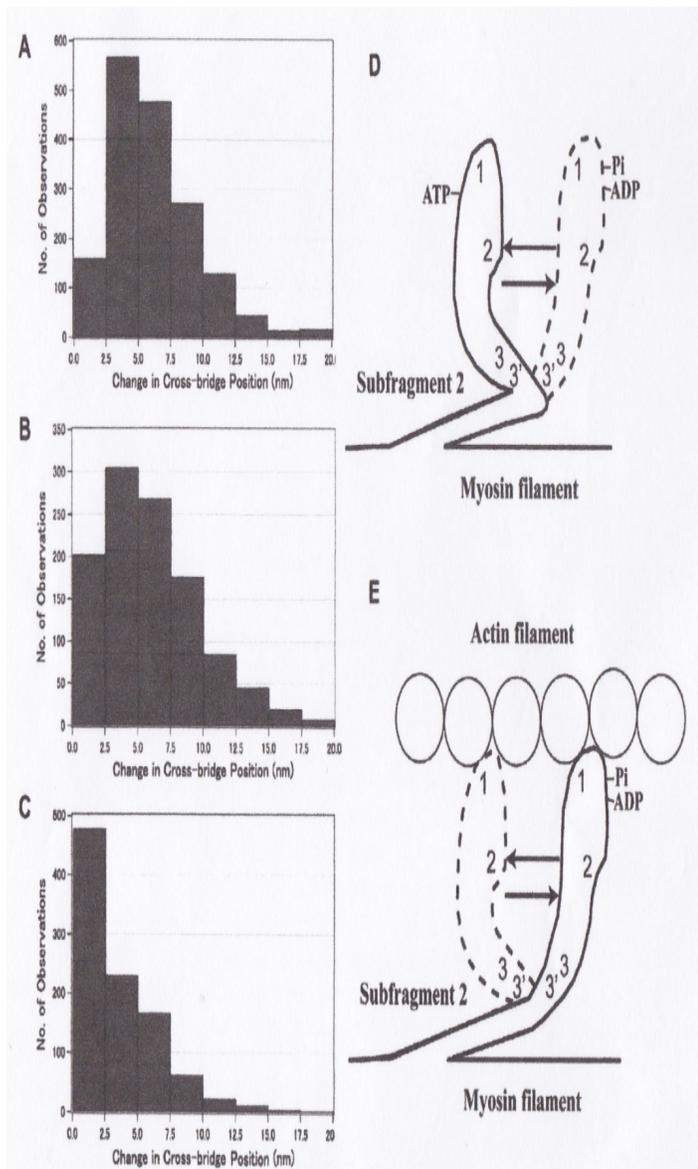
Figure 9: Amplitude of ATP-induced recovery stroke at different regions of myosin heads in hydrated myosin filaments, as recorded using anti-CAD, anti-COD, and anti-LD antibodies. (A, B and C) Histograms of amplitude distribution of ATP-induced myosin head recovery stroke in the absence of actin filaments. Myosin heads were position-marked by anti-CAD antibody in (A), anti-COD antibody in (B), and anti-LD antibody in (C). Note that the average amplitude is the same between (A) and (B). (D) Diagram illustrating myosin head recovery stroke in the absence of actin filament. (E) Diagram illustrating putative power stroke based on the result in (D) [18].
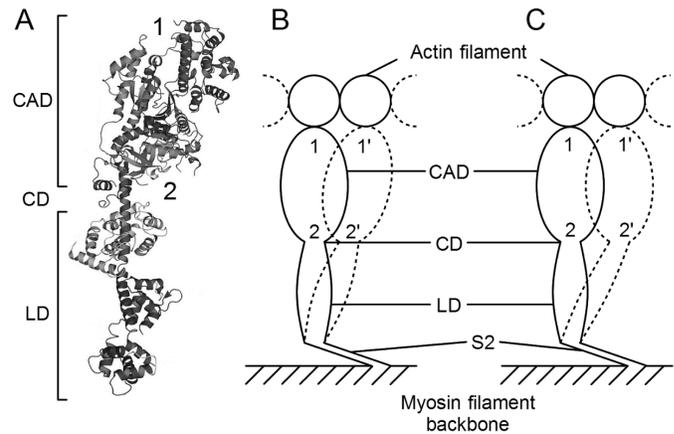

Figure 10: Two different modes of myosin head power stroke in hydrated myosin filaments, obtained from experiments using the gas environmental chamber [19]. Approximate regions of attachment of anti-CAD antibody and anti-COD antibody are indicated by numbers 1,1' and 2,2', respectively. (A) myosin head structure similar to that shown in Figure 1. (B) Myosin head power stroke in the nearly isometric condition at standard ionic strength. Note that the amplitude of movement is larger at the distal region of CAD than at COD, contrary to the prediction of swinging lever arm hypothesis. (C) Myosin head power stroke in the nearly isometric condition at low ionic strength. Note that myosin head CAD moves parallel to actin filament [19].

Conclusion

Though I appreciate efforts of people in preparing slime mold myosin head crystals, and in studying their nucleotide-dependent structural changes with interesting results, the swinging lever arm hypothesis contains a number of shortcomings caused by complete ignorance of important publications indicating the essential role of myosin S-2 by its close communication with myosin head CAD, where actin-myosin interface is formed. The papers cited in this article are all published in mainstream journals, so that there is no excuse for investigators to overlook them. Nevertheless, Holmes stated in 1979 that all results support the swinging lever arm hypothesis so nicely, and therefore the hypothesis is significant and it has become the textbook norm [23]. Sir Andrew Huxley, however, criticized the Holmes's statement as follows [24]: "Perhaps so, but results that agree with one's preconceptions need to be scrutinized with especial care". Our article indicates that Sir Andrew's criticism is entirely correct. Muscle is still filled with a number of undiscovered mysteries. Much more experimental work is necessary to reach full understanding of muscle contraction at the molecular level.

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