
Evidence for the Essential Role of Myosin Head Lever Arm Domain and Myosin Subfragment-2 in Muscle Contraction

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1. Introduction

It has been well established that muscle contraction results from relative sliding between the thick and thin filaments [1,2]. Since the myofilament sliding is coupled with ATP hydrolysis, physiological function of muscle is to convert energy derived from chemical reactions into mechanical work and heat. The thick filament consists mainly of myosin, which is a large molecule (MW 450,000) with two pear-shaped “heads” and a “rod” of 156nm long. As illustrated in Fig.1A, a myosin molecule is split by tryptic digestion into two parts: (1) a rod of 113nm long, known as light meromyosin (LMM), and (2) the rest of the myosin molecules, containing the two heads and a rod of 43nm long, known as heavy meromyosin (HMM). HMM is further digested into two separate heads, known as subfragment-1 (S-1), and the rod, known as subfragment-2 (S-2). When myosin molecules polymerize to form the thick filament, LMM aggregates to constitute the filament backbone, which is polarized in opposite directions on either side of the central region, while the S-1 heads extend laterally from the filament backbone with an axial interval of 14.3nm, except for the central region where the S-1 heads are absent and called bare zone (Fig.1B). The S-2 rod is believed to serve as a “hinge” between the S-1 head and the filament to enable the head to swing away from the filament.

On the other hand, the thin filament consists primarily of two helical strands of globular actin monomers (G-actin, MW 41,700), which are wound around each other with a pitch of 35.5nm. The axial separation of G-actin in the filament is 5.46nm (Fig.1C). In vertebrate skeletal muscle, the thin filament also contains tropomyosin and troponin. The tropomyosin molecule is rod shaped, and lies in the grooves between the G-actin strands, while troponin

molecule is located in every pitch of the strands. In relaxed muscle, interaction between the S-1 heads and the thin filament is inhibited by tropomyosin. When Ca^{2+} binds to troponin, it removes the inhibitory effect of tropomyosin to start muscle contraction [3].

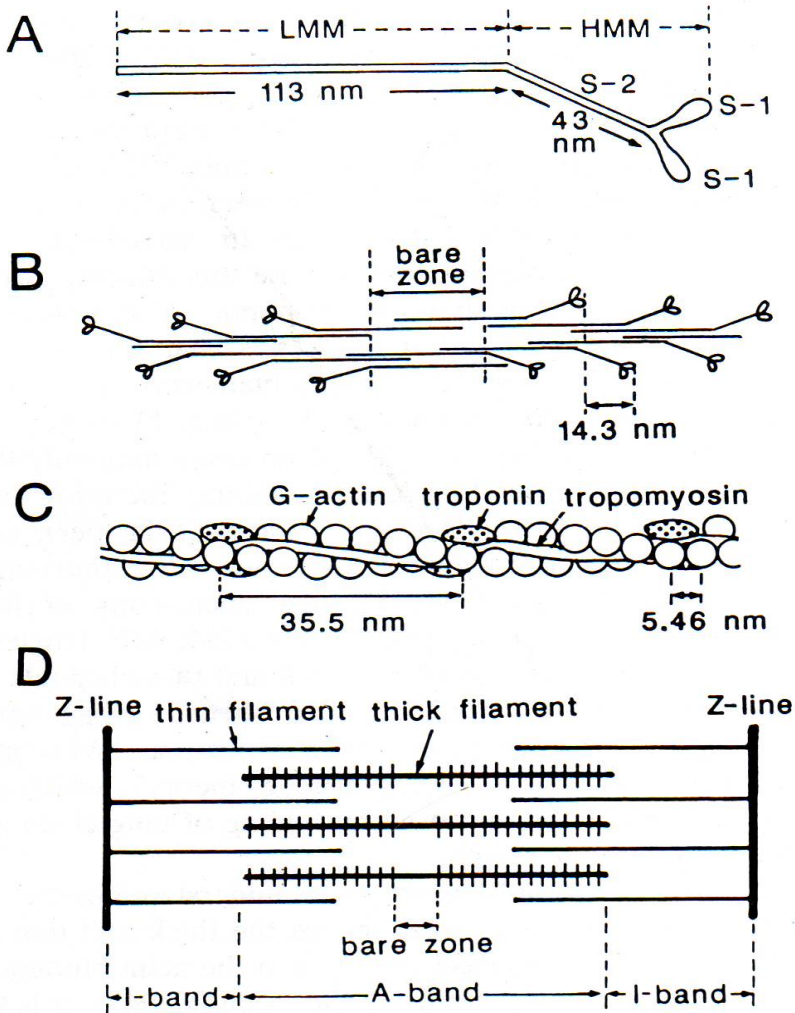


Figure 1. Structure of the thick and thin filaments and their arrangement within a sarcomere. **A** Diagram of a myosin molecule. **B** Longitudinal arrangement of myosin molecules in the thick filament. **C** Structure of the thin filament. **D** Longitudinal arrangement of the thick and thin filaments within a sarcomere. Note that the half-sarcomere is the structural and functional unit of muscle.

A skeletal muscle fiber is composed of longitudinally repeated structural units, called sarcomeres, which are bounded by Z-lines. As diagrammatically shown in Fig.1D, the thin filaments extend in either direction from the Z-line to penetrate in between the thick

filaments, located centrally in each sarcomere [3]. The region containing only the thin filaments is called the I-band, while the region containing the thick filaments as well as part of the thin filaments is called the A-bands. It has been firmly established that the filament lengths remain constant during active contraction and passive stretch of muscle fibers, based on (1) light microscopy of muscle fibers and myofibrils [1,2]; (2) electron microscopy of the filaments [4]; (3) X-ray diffraction of muscle fibers, in which various periodicities of the filaments can be measured [5]. These findings constitute evidence for the sliding filament mechanism of muscle contraction, which appears in every textbook in physiology.

Thus, the central problem in understanding molecular mechanism of muscle contraction is: what makes the thick and thin filaments to slide past each other? Since the ATPase activity and the actin binding site are located in the S-1 heads of myosin molecule, it is generally believed that the myosin S-1 heads, extending from the thick filaments towards the thin filaments, may play an essential role in the chemo-mechanical energy conversion taking place in contracting muscle.

2. Theories of myofilament sliding producing muscle contraction

Since the lengths of the thick and thin filament remain unchanged before, during and after the myofilament sliding, i.e. muscle contraction, it seems natural to consider that the myofilament sliding is caused by cyclic formation and breaking of linkages between the S-1 heads on the thick filaments and the corresponding sites on the thin filaments. The cyclic interaction between the S-1 head and the thin filament is obviously coupled with ATP hydrolysis. Most theories about mechanism of muscle contraction have been based on this idea.

2.1. Attachment-detachment cycle between the S-1 head on the thick filament and the sites on the thin filaments

Fig. 2 shows diagrams illustrating hypothetical attachment-detachment cycle between the myosin S-1 head and the corresponding site on the thin filaments, put forward by H.E.Huxley [6]. In each diagram, three rectangular-shaped S-1 heads extend from the thick filament upwards to face the sites on the thin filament, represented by small rectangular projections. Left S-1 head first attaches to the site on the thin filament, which happened to be in its close vicinity (top), changes its configuration to move the thin filament to the right (arrow, middle), and then detach from the site on the thin filament (bottom). As the result, another site on the thin filament comes in close vicinity of right S-1 head, which then starts the cycle again. Axial spacing of the S-1 heads on the thick filament differs from that of the sites on the thin filament, so that the attachment-detachment cycle takes place asynchronously. Up to the present time, the attachment-detachment cycle shown in Fig.2, still constitutes the framework of most contraction models at the molecular level. The most crucial step of the attachment-detachment cycle is, of course, conformational changes of the S-1 head attached to the thin filament shown in the middle diagram.

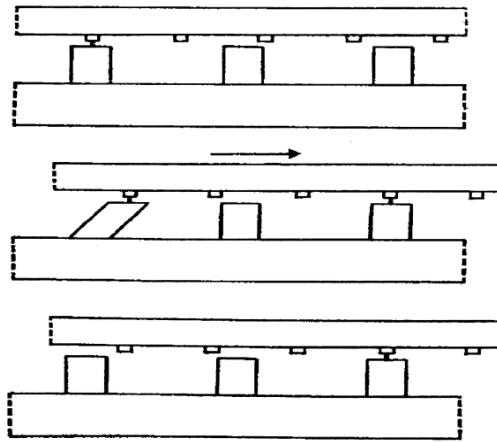


Figure 2. Diagrams of putative attachment-detachment cycle between the S-1 head, extending from the thick filament, and the corresponding site on the thin filament. For explanations, see text.

2.2. Actomyosin ATPase reaction

Molecular mechanism of muscle contraction can be studied biochemically by examining ATPase reaction steps of actin-myosin complex (actomyosin) in solution. To relate the enzyme kinetics of actomyosin in solution, the following limitations should be kept in mind: (1) the fundamental function of muscle to convert energy from chemical reactions into mechanical work can not be studied on actomyosin in solution; (2) no theories exist to directly relate the enzyme kinetics of actomyosin to the cyclic interaction between the thick and thin filaments in muscle; and (3) to study the enzyme kinetics in solution, fragments of myosin molecule (HMM or S-1) should be used at concentrations $< \text{several } \mu\text{M}$, while the effective concentration of myosin molecule and myosin S-1 head in muscle are $> 100 \mu\text{M}$.

The most probable sequence of actomyosin ATPase reaction taking place in contracting muscle is shown in Fig.3 [7,8]. M and A represent the myosin S-1 head on the thick filament and the actin monomer on the thin filament, respectively. The reaction cycle includes attachment of A to, and its detachment from M, thus providing a simple correspondence with the attachment-detachment cycle between actin and myosin in the Huxley contraction model shown in Fig.2, the above actomyosin ATPase reaction scheme is, therefore, believed to be applicable to the attachment-detachment cycle between the S-1 heads and the thin filaments in muscle.

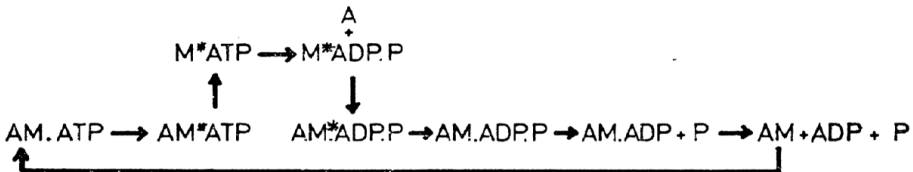


Figure 3. The most probable sequence of actomyosin ATPase reactions taking place in contracting muscle.

2.3. The myosin S-1 head tilting model

As illustrated in Fig.2, the S-1 head, attached to the thin filament, should undergo structural changes to cause relative sliding between the thick and thin filaments. Although a number of attempts have been made up to the present time using a variety of experimental methods, the question, as to what makes the filaments slide, is not yet fully answered. In 1977, A.F.Huxley and Simmons presented a contraction model, which was central in the field of muscle physiology over many years [9](Fig.4). The essence of this model is summarized as follows: during muscle contraction, each S-1 head first attaches to the thin filament (A→B), changes its angle of attachment to the thin filament from 90° to 45°, while attached to the thin filament (B→C), thus producing a unitary step for filament sliding, and then detach from the thin filament (D). In this contraction model, the S-2 only serves to transmit force generated by the “tilting” S-1 head to the thick filament backbone. A number of investigators made experiments to prove the “S-1 head rotation”, including the use of fluorescent ATP and ADP analogs [10] and spin labels [11] attached to the S-1 head, with results that the change in S-1 head orientation was very limited during muscle contraction. Time-resolved X-ray diffraction studies on contracting muscle also could not detect any appreciable changes in the equatorial reflections in response to a quick decrease in muscle length, which was expected to synchronously rotate the massive S-1 head [12,13]. Thus, the myosin S-1 head tilting model was found to be inconsistent with the experimental observations.

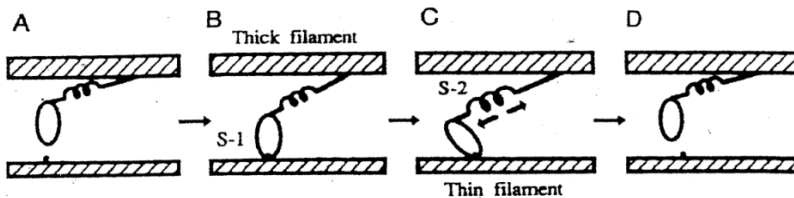


Figure 4. Diagram showing the attachment-detachment cycle between the S-1 head and the thin filaments, presented by A.F. Huxley and Simmons [10]. For explanations, see text.

Since the S-1 head tilting model is not supported experimentally, the S-1 head structural changes are considered to be limited within a small region in the myosin molecule.

2.4. The swinging lever arm hypothesis

The X-ray S-1 head crystal structure was first obtained by Rayment et al.[15] on chicken skeletal muscle. As shown in Fig.5, the S-1 head structure is tadpole-like in shape, with an elongated head containing the catalytic domain (CD) consisting of 25K (green), 50K (red) and part of 20K (dark blue) fragments of myosin heavy chain, and a tail, i.e. the lever arm domain (LD) consisting of the rest of 20K fragment and the essential (ELC, light blue) and the regulatory (RLC, magenta) light chains. The CAD and the LD are connected via the converter domain (COD). In the intact myosin molecule, the LD is connected to the thick filament via the myosin S-2. Attempts have been made to study possible nucleotide-dependent structural changes of the S-1 head to obtain insight into the mechanism of

muscle contraction. For this purpose, the S-1 head is truncated at its tail except for the COD, thus eliminating the LD including the ELC and the RLC. The truncated S-1 is nearly globular in shape, and is easy to crystallize. It is regarded as “minimal motor” to obtain information about muscle contraction mechanism, though the validity to eliminate and ignore possible function of the LD and the S-2 in muscle contraction is not proved up to the present time.

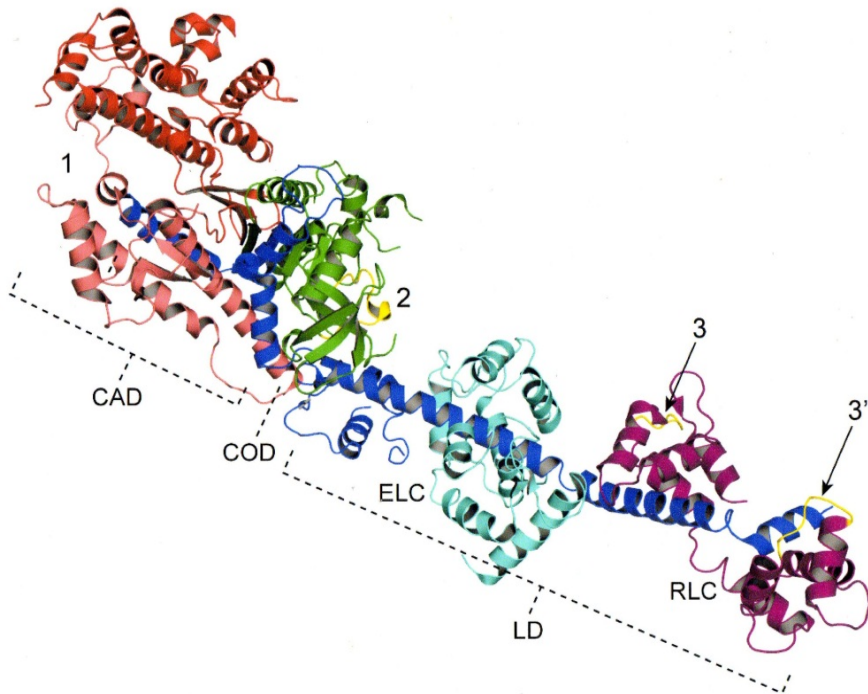


Figure 5. Myosin S-1 head structure [15]. For explanations, see text. Location of peptides around Lys 83 and that of two peptides (Met 58~Ala 70 and Leu 106~Phe 120) in the LD are colored yellow. Numbers 1, 2 and 3,3' indicate approximate regions of attachment of antibodies 1, 2 and 3, used by Sugi et al., respectively [14]. For further explanations, see text. Figure prepared by using software PyMOL (<http://pymol.sourceforge.net>).

The crystal structures of truncated myosin obtained from a slime mold *Dictyostelium* have been studied with various ATP analogs, including ADP · BeF₃, ADP · AlF₄, and ADP · vanadate, and it has been shown that the truncated S-1 takes two different structures depending on the kind of ATP analogs; between the two states, the COD rotates by about 60° [for a review, see15]. Based on this and other results, it has been proposed that the power stroke of the S-1 head, causing myofilament sliding, results from active rotation of the CD around the COD [15], utilizing chemical energy of ATP hydrolysis taking place in the CD. This is the swinging lever arm hypothesis, which now appears in many textbooks in physiology and biology.

It is not certain, however, whether the above nucleotide-dependent structural changes of the S-1 head actually work in muscle contraction or not by the following reasons: (1) It is not clear that the observed rotation of the COD generates torque large enough to cause the filament sliding when the COD is connected to the thick filament via the LD and S-2; (2) It seems possible that the rotation of the COD is an artifact arising from close packing of the S-1 in the crystal, that may make each S-1 in a condition completely different from that in muscle; (3) It seems also possible that the ATP analogs used do not actually mimic intermediate compounds of ATP hydrolysis in muscle; and (4) The hypothesis completely ignores possible roles of the LD as well as the S-2.

2.5. Power and recovery strokes of the S-1 head coupled with ATP hydrolysis

Fig. 6 illustrates the most plausible attachment-detachment cycle between the S-1 head (M), extending from the thick filament and actin monomer (A) in the thin filament, based on the actomyosin ATPase reaction shown in Fig.3 [7,8]. First, M in the form of complex, $M \cdot ADP \cdot Pi$, attaches to A (A), and exerts a power stroke, associated with release of Pi and ADP (from A to B). After the end of power stroke, M remains attached to A (B). Upon binding with ATP, M detaches from A, and exerts a recovery stroke, associated with reaction, $M \cdot ATP \rightarrow M \cdot ADP \cdot Pi$ (from C to D). Then, $M \cdot ADP \cdot Pi$ again attaches to A (from D to A), and the cycle is repeated. In this diagram, both the power and the recovery strokes of M is supposed to result from the swinging lever arm mechanism, so that M does not change its angle of attachment to A throughout the whole cycle, while it swings around a pivot (COD, represented by a small circle) forward and backward. The LD is located between M and the pivot, simply serving as a lever arm.

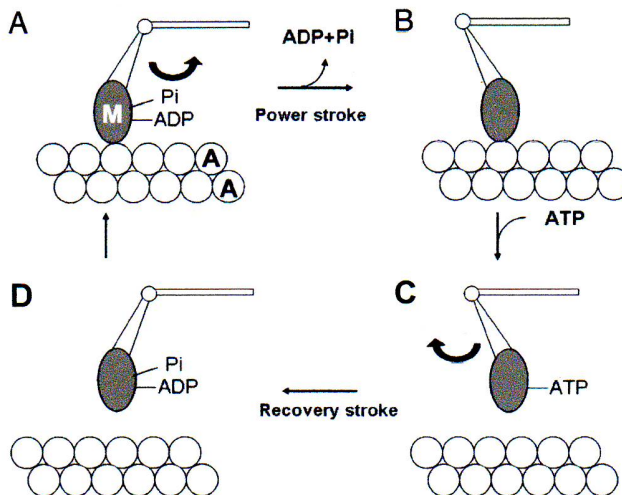


Figure 6. Diagram showing the attachment-detachment cycle between myosin S-1 head (M), extending from the thick filament, and actin monomer (A) in the thin filament, based on the actomyosin ATPase reactions [16]. For explanations, see text.

It is understandable that crystallographists use truncated S-1 head because of easiness in crystallizing it. Another reason for their ignorance of the LD and the S-2 in considering the mechanism of muscle contraction may come from development of so-called *in vitro* motility assay experiments, in which fluorescently labeled actin filaments are made to slide over myosin molecules or their proteolytic fragments such as HMM and S-1, fixed on a glass surface in the presence of ATP [17]. Especially, the fact that even the S-1 alone can generate force on actin filaments [18] seems to have given muscle investigators a belief that only the S-1 head is important in producing muscle contraction.

Fig.7 is a more realistic diagram showing the myosin S-1 head power stroke based on the swinging lever arm mechanism, in which the myosin S-1 head power stroke is accompanied by swing of the SD around the COD and also swing of the LD around the boundary between the LD and the S-2, while the CD structure remains unchanged before (solid line) and after (broken line) the power stroke. We think that there is no reason to ignore the LD-S-2 boundary in considering the mechanism of the myosin S-1 head power stroke. In the next section, we will present experimental evidence for the essential role of the LD and the S-2, as well as evidence for non-essential role of the COD.

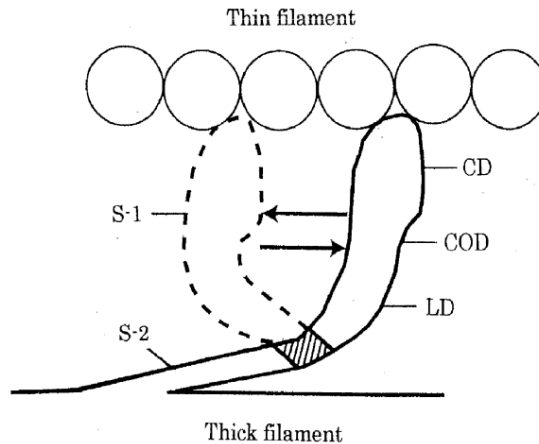


Figure 7. Diagram showing the structural changes of the myosin S-1 and S-2 before (solid line) and after (broken line) the power stroke. Note that the S-1 swings around the boundary between the S-1 LD and the S-2 (shaded area) in addition to the CD swing around the COD).

3. Evidence for the essential role of the S-1 head lever arm domain (LD) and the myosin subfragment-2 (S-2) region in muscle contraction

Our experiments concerning the essential role of the myosin S-2 started in 1992 when one of us (H.S.) was asked by the late Professor Harrington of Johns Hopkins University to work with him using a polyclonal antibody directed to the myosin S-2 (anti-S-2 antibody). At that time, Harrington had a unique idea that, in addition to the S-1 head power stroke, shortening of the S-2 region resulting from α -helix to random coil transition within a limited part of S-2 [19,20].

3.1. Effect of anti-S-2 antibody on the contraction characteristics of Ca²⁺-activated rabbit psoas muscle fibers

Single glycerol-extracted muscle fibers, prepared from rabbit psoas muscle, were maximally activated with 10⁻⁴M Ca²⁺ in contracting solution before and at various times after administration of anti-S-2 antibody (1.5mg/ml). Force-velocity (P-V) curves were determined by applying ramp decreases in force at the plateau of Ca²⁺-activated isometric force with a servo-motor. Muscle fiber stiffness was measured by applying small sinusoidal length changes (1 kHz, peak-to-peak amplitude ~0.1% of fiber length) and recording resulting force changes [21].

The magnitude of Ca²⁺-activated isometric force in the fibers decreased with time in the presence of antibody, while the maximum unloaded velocity of shortening V_{max} remained unchanged, as shown by the P-V curves in Fig.8A [21]. If the velocity values are replotted against forces expressed relative to steady forces, the P-V curves were found to be identical (Fig.8B). Since muscle fiber stiffness changed in parallel with force (Fig.9), the decrease in force is due to decrease in the number of myosin S-1 head involved in force generation, and myosin S-1 heads that stop interacting with the thin filament do not provide internal resistance against fiber shortening [21].

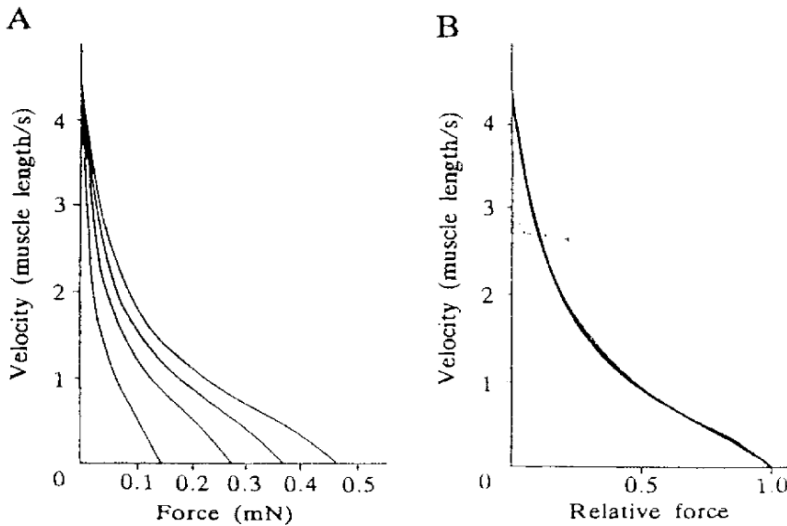


Figure 8. Effect of anti-S-2 antibody on P-V relation in a Ca²⁺-activated single muscle fiber. (A) P-V curves obtained before (control) and 30, 60, and 90min after administration of anti-S-2 antibody. Both velocities and forces are expressed in absolute values. Note that the maximum shortening velocity remains unchanged despite marked reduction of isometric force. (B) The same P-V curves in which forces are expressed relative to their respective steady forces. Note that the curves are identical in shape [21].

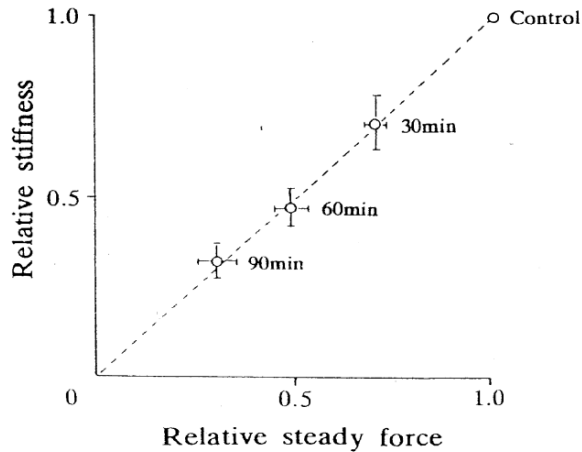


Figure 9. Stiffness versus force relation at steady Ca^{2+} -activated isometric forces of single muscle fibers before (control) and 30, 60 and 90min after administration of anti-S-2 antibody (1.5mg/ml). Each data point represents the mean of seven different experiments. Vertical and horizontal bars indicate SD of stiffness and force, respectively [21].

3.2. Effect of anti-S-2 antibody on MgATPase activity of Ca^{2+} -activated muscle fibers

MgATPase activity of Ca^{2+} -activated muscle fibers was recorded by decrease of NADH during cleavage of ATP [21]. A small fiber bundle consisting of 2–3 fibers was mounted in the sample compartment of a dual-wavelength spectrophotometer (model 156, Hitachi) with a sample monochromator at 340nm and a reference monochromator at 400nm. Examples of simultaneous recordings of MgATPase activity and isometric force development in the fibers are shown in Fig. 10. It was found that the MgATPase activity, judged from the slope of the ATPase records, did not change appreciably even when the force development was completely eliminated, indicating a complete dissociation of force development from MgATPase activity. This implies that, anti-S-2 antibody inhibits Ca^{2+} -activated force development by impairing the function of the S-2 region in producing the myosin head power stroke.

A question arises as to how the chemical energy of ATP hydrolysis, taking place in the myosin head CD can be transmitted to the S-2 region, distant from the CD region. Kobayashi et al. [22] have presented results suggesting that the myosin head CD can communicate with the myosin S-2. If anti-S-2 antibody is applied to the fiber after development of the isometric force, both the force and the stiffness decrease in parallel with each other with time. If, on the other hand, anti-S-2 antibody is applied to the fiber after development of rigor force in the absence of ATP, it shows no effect on both rigor force and stiffness. If it is assumed that the S-2 participates not only in Ca^{2+} -activated force development in the presence of ATP, but also in rigor force development in the absence of

ATP, then these results suggest that anti-S-2 antibody binds with the S-2 when the myosin head CD is interacting cyclically with the thin filament, but not when the CD stops cyclic interaction with the thin filament due to permanent rigor linkage formation.

Meanwhile, our recent unpublished experiments have indicated that a monoclonal anti-LD antibody, which attaches to the regulatory light chain close to the LD-S-2 boundary, inhibits Ca^{2+} -activated muscle fiber contraction. This, together with the inhibitory effect of anti-S-2 antibody on muscle fiber contraction, strongly suggests the essential role of the LD-S-2 boundary in muscle contraction.

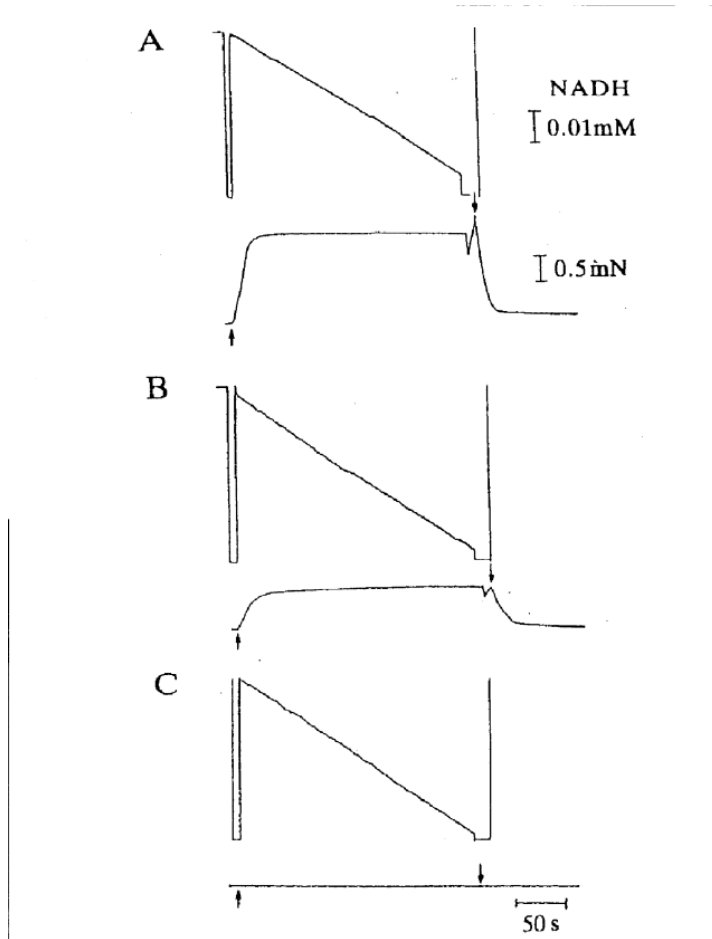


Figure 10. MgATPase activity (upper traces) and Ca^{2+} -activated isometric force (lower traces) in a small fiber bundle, before (A), at 100min (B) and at 150min (C) after application of antibody. Note that the slope of ATPase records does not change appreciably even when the force is reduced to zero. Times of application of contracting and relaxing solutions are indicated by upward and downward arrows, respectively [21].

3.3. Evidence against the essential role of the myosin head converter domain (COD) in muscle contraction

Muhlrad et al. [23] reported that chemical modification (trinitrophenylation) of reactive lysine residue (RLR), located close to the myosin head converter domain (COD) inhibited both actin-activated ATPase activity of RLR-modified S-1 head and in vitro actin filament sliding over RLR-modified myosin heads. They interpreted these results as being due to steric clashes between the modified RLR and the CVD structure to inhibit the myosin head power stroke as well as the ATPase activity in the myosin head CD. Their results seem to indicate the essential role of myosin head COD in muscle contraction,

Using the gas environmental chamber, which enables us to study dynamic structural changes of hydrated biomolecules retaining their physiological function, we succeeded in recording ATP-induced movement of myosin S-1 heads (mean amplitude, ~6nm) extending from hydrated synthetic thick filaments [16,24]. To position-mark individual S-1 heads in unstained filament samples, we used a monoclonal antibody directed to junctional peptide between 50K and 20K fragments of myosin heavy chain (antibody 1 in Fig.5).

Also using the gas environmental chamber, we could record ATP-induced movement at three different parts within individual S-1 heads with three different site-directed antibodies; antibody 1, antibody 2 to reactive lysine residue (RLR) located close to the COD, and antibody 3 to two peptides in the regulatory light chain in the LD (Fig.5) [14]. The results obtained are summarized in Fig.11. It was found that the mean amplitude of ATP-induced movement was ~6nm both around the distal end of the CD (Fig.11) and around the COD (Fig.12B), and ~3.5nm around the proximal end of the LD (Fig.11). As our experiments were made in the absence of actin filaments, we recorded the recovery stroke of individual S-1 heads, coupled with reaction, $M \cdot ATP \rightarrow M \cdot ADP \cdot Pi$, corresponding to the steps C to D in Fig.6 (Fig.12D). The S-1 head recovery stroke is believed to be the same in amplitude as, but opposite in direction to, the S-1 head power stroke (corresponding to the steps A to B in Fig.6) (Fig.12E).

We were interested in the fact that individual S-1 heads, position-marked with antibody 2, which attaches to RLR close to the COD, still exhibit ATP-induced movement, because it indicated that attachment of massive antibody (IgG) to RLR does not cause any steric clashes to inhibit S-1 movement. To solve the above puzzling result, we have examined the effect of anti-RLR antibody (=antibody 2) on both in vitro ATP-dependent sliding of actin filaments over myosin molecule fixed to a glass surface and Ca^{2+} -activated muscle fiber contraction [25]. Application of anti-RLR antibody inhibited in vitro actin filament sliding over myosin, being consistent with the result of Muhlrad et al. [23] that chemical modification of RLR inhibits in vitro actin filament sliding over myosin. Unexpectedly, however, anti-RLR antibody had no effect on muscle fiber contraction [25]. Though our research work is still in progress, the results stated above, together with our unpublished observations, can be taken to imply that (1) the swing of the CD around the COD, suggested by the swinging lever arm hypothesis may not be an active process, and may not be essential in producing filament sliding taking place in the hexagonal filament-lattice in muscle.

Much more experimental work on the role of myosin head LD and myosin S-2 is necessary for full understanding of the mechanism of muscle contraction at the molecular and submolecular levels.

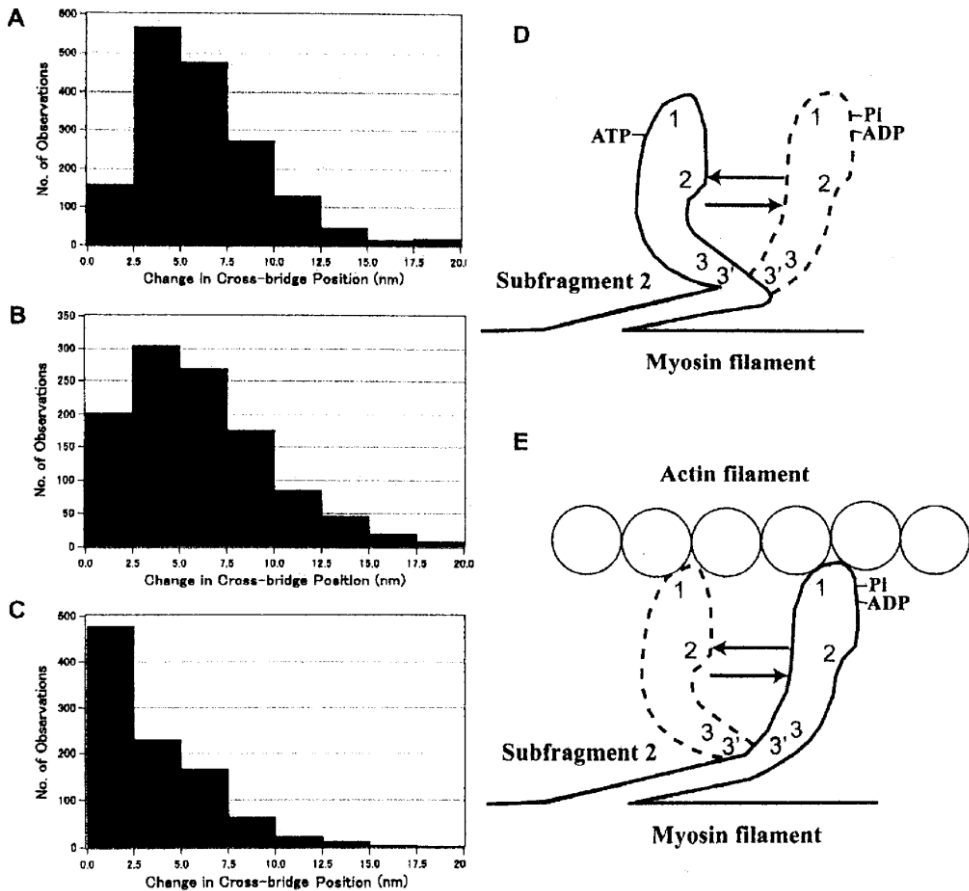


Figure 11. (A–C) Histograms of the amplitude distribution of ATP-induced myosin S-1 head movement, position-marked with antibody 1 (A), antibody 2 (=anti-RLR antibody)(B), and antibody 3 (=anti-LD antibody)(C). (D, E) Diagrams showing possible changes in shape of myosin S-1 head during the recovery stroke in the absence of actin filament (D) and during the power stroke in the presence of actin filament (E). Approximate regions of attachment of antibodies 1, 2 and 3, 3', respectively [14].

4. Summary

Muscle contraction results from relative sliding between the thick (myosin) and thin (actin) filaments, which in turn is caused by the attachment-detachment cycle between the myosin heads extending from the thick filaments and the actin monomers in the thin filaments. The myosin heads (myosin subfragment 1,S-1) consists of the catalytic domain (CD) and the lever arm domain (LD), which are connected via the converter domain (COD). The S-1 is connected to the thick filament via myosin subfragment 2 (S-2). It is generally believed that each S-1 head undergoes structural changes (power stroke) while attached to actin, thus producing unitary filament sliding.

The swinging lever arm hypothesis, which assumes active rotation of the CD around the COD, is constructed on the basis of crystallographic studies on the truncate S-1, in which both the LD and the S-2 are eliminated, and therefore completely ignores possible role of the LD and the S-2. In this article, we presented evidence for the essential role of the LD and the S-2, as well as evidence against the swinging lever arm hypothesis. We emphasize that, to reach full understanding of muscle contraction mechanism, much more experimental work is necessary using experimental systems, in which connection of the S-1 to the thick filament remains intact.

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