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Effect of Antibodies to Myosin Head on the Development of Rigor Tension and Stiffness in Skinned Muscle Fibers

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Abstract

Using three antibodies to myosin head, attaching to (1) distal region and (2) proximal region of myosin head catalytic domain, and (3) to myosin head lever arm domain, respectively, we have shown definite differences between *in vitro* actin-myosin sliding and muscle contraction. In the present study, we studied the effect of these antibodies on the development of rigor tension and stiffness in single skinned muscle fibers at pCa>9. To form rigor actin-myosin linkages, myosin heads should override tropomyosin, covering myosin-binding sites on actin, and to detach antibodies from them. Despite their different attachment sites in myosin head, all these antibodies slowed down development of rigor tension and stiffness with or without changing their peak values. The rigor tension versus stiffness relation was highly variable, suggesting that rigor tension reflects the sum of tension in individual rigor linkages, while the rigor stiffness represents the total number of rigor linkages. Dummy antibody had no effect on the development of rigor state. These results indicate that the action of myosin heads overriding tropomyosin is inhibited by the antibodies, so that development of rigor state is slowed down due to gradual detachment of the antibodies from individual myosin heads.

Highlights

• The effect of three antibodies, attaching to different regions in myosin head's on the development of rigor state was examined at pCa >9, using single skinned muscle fibers.

• Despite their different binding sites on myosin, all the antibodies slowed down development of rigor tension and stiffness with or without changes in their peak values.

• The rigor tension versus stiffness relation was highly variable, suggesting that rigor tension reflects the sum of tension generated by individual myosin heads, while stiffness serves as a measure of total number of rigor linkages.

• These results indicate that the antibodies inhibit myosin head movement to override tropomyosin, and detachment of the antibodies from myosin heads is necessary prerequisite for rigor linkage formation.

Keywords: Muscle contraction; Rigor tension; Rigor stiffness; Antibodies to myosin head; Skinned muscle fiber

Introduction

Muscle contraction results from cyclic attachment-detachment between myosin heads extending from myosin filaments and corresponding sites in actin filaments [1]. A myosin molecule can be divided into a long rod, called light meromyosin (LMM) and the rest of the molecule, called heavy meromyosin (HMM) consisting of a short rod (myosin subfragment-2, S-2) and two pear-shaped heads (myosin subfragment-1, S-1). In myosin filaments, LMM aggregates to from filament backbone, while the two S-1 heads, which will hereafter be called myosin heads, extend laterally from myosin filaments. Muscle is regarded as a machine converting chemical energy derived from ATP hydrolysis into mechanical work. Based on the extensive biochemical studies on actin and myosin extracted from muscle [2], it is generally believed that myosin head (M) first attaches to actin (A) in the form of M-ADP-Pi to perform power stroke, associated with reaction, A-M-ADP-Pi → A-M + ADP + Pi. In this scheme, A-M is a high-affinity rigor complex in the absence of ATP. Despite a great gap between muscle contraction and biochemical experiments on extracted protein samples [3], myosin heads in contracting muscle are also generally regarded to pass through rigor state A-M.

A myosin head is composed of distal catalytic domain (CAD) and proximal lever arm domain (LD), which are connected by small converter domain (COD). Based on crystallographic and cryo-electron microscopic studies on extracted protein samples, it is also generally believed that myosin head power stroke is caused by active rotation of CAD around COD (swinging lever arm hypothesis) [4]. To examine the validity of the above hypothesis, we used three different antibodies to myosin head [5,6]; antibody 1 to junctional peptide between 50K and 20K segments of myosin heavy chain in the CAD, antibody 2 to reactive lysine residue in the COD, and antibody 3 to two light chains in the LD. We found that (1) antibodies 1 and 2 had no appreciable effect on Ca^{2+}-activated contraction of skinned muscle fibers, while antibody 3 inhibited Ca^{2+}-activated contraction in a dose-dependent manner without changing MgATPase activity [7]. These results may be taken to indicate that (1) during muscle contraction, myosin heads do not pass through rigor state AM, (2) muscle contraction may not result from active rotation of the CAD around the COD, and (3) myosin head LD play an essential role in muscle contraction, together with myosin subfragment-2 region connecting myosin heads to myosin filament backbone [8].

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As a first step to obtain further information about actin-myosin interaction in the myofilament-lattice structures in muscle, the present experiments were undertaken to study the effect the three antibodies on the formation of rigor A-M linkages between myosin heads and actin filaments in skinned muscle fibers. In relaxing solution (pCa>9), myosin heads are inhibited to interact with actin filaments by tropomyosin molecules, which wound around actin filaments to cover myosin-binding sites in actin filament [9]. Consequently, in ATP-free rigor solution, myosin heads have to override tropomyosin to form rigor linkages with actin filaments. Here we show that, despite their different binding sites in myosin head and different effects on muscle contraction and ATP-dependent *in vitro* actin-myosin sliding [7], all the three antibodies showed a qualitatively similar effect on the development of rigor state; i.e. to slow down the rate of development of rigor tension and stiffness with or without changing their peak values.

Materials and Methods

Skinned muscle fiber preparation and experimental setup

White male rats (Japan White, Sanko Lab. Industry) were killed on their delivery to our laboratory by sodium pentobarbital injection (50 mg/kg) into ear vein, and psoas muscles were dissected from the animals. The animals were treated following the Guiding Principles for the Care and Use of animals in the Field of Physiological Sciences, published by the Physiological Society of Japan. The protocol was approved by the Teikyo University Animal Care Committee (protocol #07-050). Chemically skinned muscle fiber strips were prepared from the psoas muscle [7,8]. Single muscle fibers (diameter, 40-60 µm) were isolated from the fiber strips, and mounted horizontally in an experimental apparatus between a tension transducer (AE801, SensoNor, Holten, Norway) and a servomotor (G-100PD, General Scanning, Watertown, MA) by glueing both ends with collodion. The servomotor contained a displacement transducer (differential capacitor) sensing the motor arm movement. Further details of the methods are described elsewhere [8]. The fiber was kept at its slack length (~3 mm) at a sarcomere length of 2.4 µm, measured with optical diffraction by He-Ne laser light. The experimental apparatus consisted of two solution compartments (volume, ~0.2 ml for each) made of anodized aluminum blocks. Exchange of solutions was made by lifting the fiber up from one compartment, and then putting it into another compartment. Relaxing solution (pCa, >9) contained 125 mM KCl, 4 mM $MgCl_2$, 4 mM ATP 4, 4 mM EGTA, and 20 mM PIPES. Rigor solution was prepared by omitting 4 mM ATP from relaxing solution. In both solutions, pH was adjusted to 7.0 by PIPES.

Recording of muscle fiber stiffness

To estimate the time required to establish rigor state after putting the fiber from relaxing to rigor solution, we recorded changes in muscle fiber stiffness by applying small sinusoidal vibrations (peak-to-peak amplitude, 0.2% of slack fiber length Lo; frequency 2 kHz) with the servomotor [8]. The tension signals consisted of tension generated by the fiber and superimposed sinusoidal component caused by applied vibration. The in-phase sinusoidal component and the (90 deg) out-of-phase component (quadrature stiffness) were separated from muscle fiber tension with a lock-in amplifier to be recorded together with tension changes in the fiber. All the experimental records were displayed and recorded on an X-Y chart recorder [8].

Experimental procedures

In control experiments, the fiber was first equilibrated in relaxing solution for 10-15 min, and then transferred into rigor solution, and subsequent development of tension and stiffness were recorded. After establishment of full rigor state, as indicated by development of tension and stiffness to steady values, the fiber was made to relax completely by returning it to relaxing solution. To examine the effect of antibodies on the development of rigor tension and stiffness, the fiber was kept in relaxing solution containing antibody 1, 2 or 3 (up to 2 mg/ml) for 20-30 min, and then put into rigor solution containing antibody (up to 2 mg/ml), and the resulting development of rigor tension and stiffness were recorded. The fiber was then made to relax in relaxing solution without antibodies. In most cases, control experiments were made before the experiments in the presence of antibody. In some cases, the sequence was reversed with similar results. Experiments were performed at 20°C unless otherwise stated.

Results

General features of rigor tension and stiffness in the absence of antibodies

As shown in Figure 1, rigor force and stiffness in skinned muscle fibers increased in parallel with each other on application of rigor solution, reaching their peak values at the same time. The development of rigor state was not influenced appreciably in the presence of dummy antibody to human C reactive protein, which has no epitopes in the fibers, indicating that antibody (IgG) molecules, not attached to myosin heads, have no appreciable effect on the development of rigor state. In 30 different fibers studied, the time from the application of rigor solution to the full development of rigor tension and stiffness showed a wide range of variation from 40 s to 3 min, while the maximum rigor tension ranged from 100-300 µN (or 20-40 kN/m^2), amounting ~50% of the maximum isometric tenson in Ca^{2+}-activated muscle fibers [10]. On returning the fiber to relaxing solution, both rigor tension and stiffness fell rapidly to zero in a few s. The development of rigor state in rigor solution was reproducible and could be repeated a few times. Despite the parallel development of rigor tension and stiffness, the slope of stiffness versus tension relation differed markedly from fiber to fiber (Figure 2). This may reflect complicated process of rigor linkage formation to be discussed later. When temperature was lowered from 20 to 0°C, peak rigor tension decreased to ~one-fourth, and the rate of development of rigor state was markedly reduced, in such a way that rigor tension and stiffness still continued to rise slowly even at 15 min after application of rigor solution, indicating a very large temperature

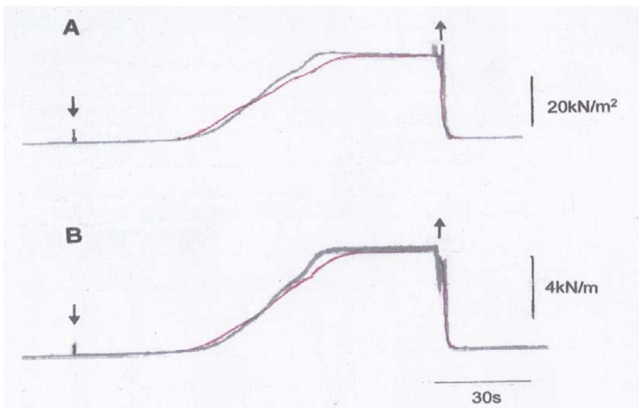

Figure 1: Development of rigor tension (A) and stiffness (B). In this and Figures 3-6, downward and upward arrows indicate times of application and removal of rigor solution, respectively. Records in the presence of dummy antibody (2 mg/ml) are colored red. Not no appreciable effect of dummy antibody on the development of rigor tension and stiffness.

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coefficient Q_{10} (probably ~4) for the rate of rigor state development (Figure 3).

Effect of antibodies 1, 2 and 3 on development of rigor tension and stiffness

Antibodies 1, 2 and 3 exhibited clear effects on the development of rigor tension and stiffness at concentrations from 1.5 to 3.0 mg/ml. Figures 4-7 are examples of records showing the development of rigor tension and stiffness in the absence and presence of antibodies 1, 2 and 3. In contrast with their different attachment sites in myosin head as well as their definitely different effects on Ca^{2+}-activated muscle contraction and ATP-dependent *in vitro* actin-myosin sliding [7], these antibodies showed a qualitatively similar effects on the development of rigor state at pCa>9. The effects can be summarized as follows: (1) The rate of development of rigor tension and stiffness, as measured from the time of application of rigor solution to the time at which rigor tension and stiffness reached their half maximum value, was markedly slowed down to one-third to one-fifth, the extent of the antibody-induced reduction of rigor tension and stiffness development being differet from fiber to fiber (Figures 4-7); (2) after reaching a peak, rigor tension started decreasing, while rigor stiffness stayed almost constant (Figures 4-7); (3) Depending on the fiber used, these antibodies affected the peak values of rigor tension Tmax and rigor stiffness Smax in three different modes; (i) both Tmax and Smax did not change appreciably (Figure 4), (ii) both Tmax and Smax decreased by >30%, and (iii) either Tmax or Smax decreased or increased and vice versa (Figures 6 and 7).

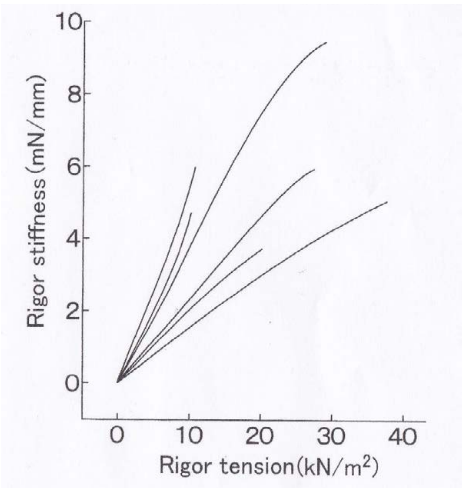

Figure 2: Relation between rigor tension and rigor stiffness, obtained during the development of rigor tension and stiffness. The tension versus stiffness curves are obtained from different muscle fibers. Note that the slope of the curve differs markedly from fiber to fiber.

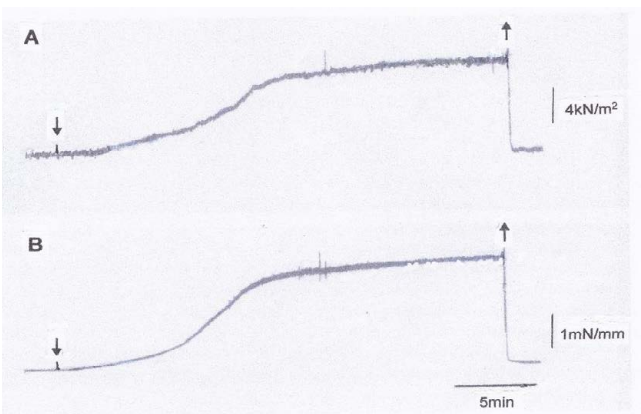

Figure 3: Development of rigor tension (A) and stiffness (B) at 0°C. Note marked reduction in the rate of development of rigor tension and stiffness.

Discussion

Possible mechanism of development of low-Ca rigor state

It is well known that, on removal of external ATP by transferring muscle fibers from relaxing to rigor solutions, the fibers are put into rigor state, in which almost all myosin heads are believed to form rigor linkages with actin [11]. The fibers can also be put into rigor state by removing external ATP from contracting solution (pCa, 4) [12]. Therefore, there are two types of rigor state, high-Ca rigor established at pCa 4, and low-Ca rigor state established at pCa>9. At low pCa (>9),

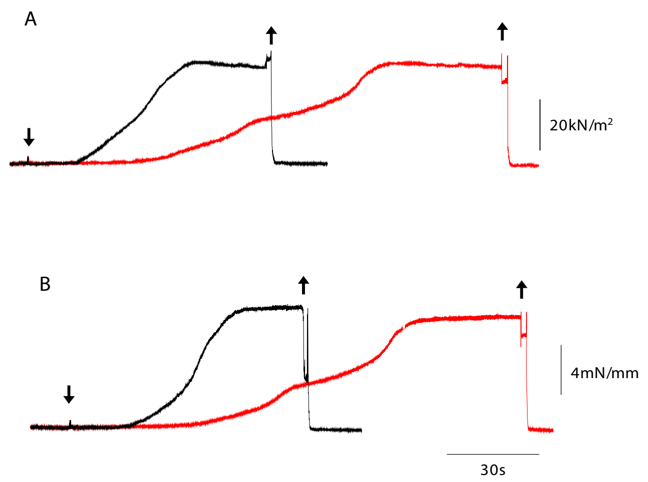

Figure 4: Effect of antibody 1 (2 mg/ml) on the development of rigor tension (A) and stiffness (B). In this and subsequent figures, records in the presence of antibody are colored red. Note that, in this particular muscle fiber, antibody 1 slows down rate of development of rigor tension and stiffness markedly, without appreciably changing their peak values.

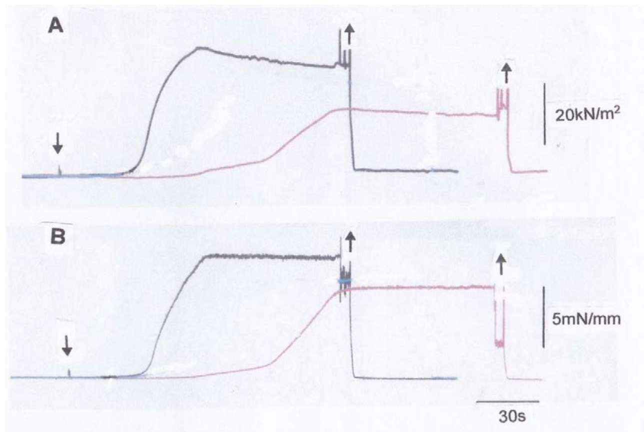

Figure 5: Effect of antibody 1 (2 mg/ml) on the development of rigor tension (A) and stiffness (B). Note that, in this muscle fiber, antibody 1 slows down rate of development of rigor tension and stiffness markedly, with marked reduction in their paek values.

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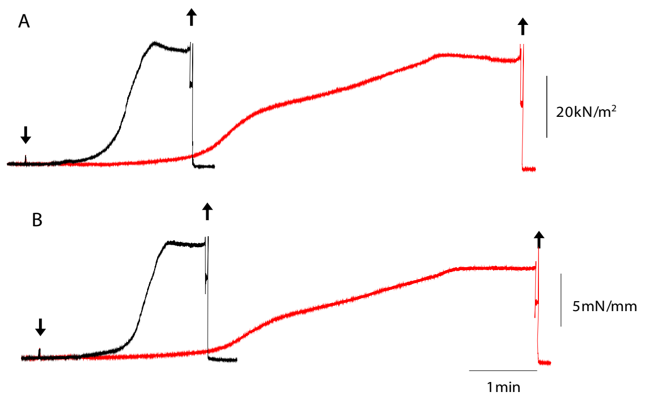

Figure 6: Effect of antibody 2 (2 mg/ml) on the development of rigor tension (A) and stiffness (B). Note that, in this muscle fiber, antibody 2 slows down rate of development of rigor tension and stiffness, with small reduction in their peak values.

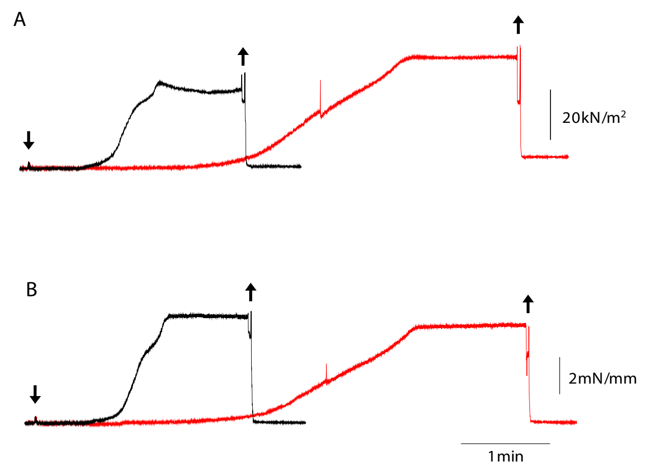

Figure 7: Effect of antibody 3 (2 mg/ml) on the development of rigor tension (A) and stiffness (B). Note that, in this fiber, antibody 3 slows down rate of development of rigor tension and stiffness, with marked increase in rigor tension.

myosin heads are inhibited to interact with actin by tropomyosin, which wind around actin filaments to cover myosin-binding sites in actin filaments [3,9]. Since the present experiments were undertaken to study the effect of antibodies to myosin heads on the formation of A-M linkages in low-Ca rigor fibers. It is necessary to consider the possible mechanism, in which myosin heads form rigor linkages with actin by overriding tropomyosin. A most plausible sequential mechanism may be stated as follows: (1) At various limited regions within the fiber, tropomyosin can be displaced relatively easily by movement of myosin heads, which tend to bind with actin; (2) As the result, myosin heads in the limited regions override tropomyosin to form rigor linkages with actin; (3) This causes further displacement of neighboring tropomyosin to form new rigor linkages; and (4) thus, the action of myosin heads to override tropomyosin gradually spreads over the whole interior of the fiber to result in establishment of rigor state, as indicated by the gradual development of rigor tension and stiffness to reach their peaks.

Diffusion of ATP into or out of the fiber has been calculated using the diffusion constant of ATP within the fiber [12,13]. According to this calculation, ATP concentration at the center of the fiber (diameter, 50 μm) is reduced below 1 μM in 10 s after applicator of rigor solution. The present result that the time required for establishment of rigor state after application of rigor solution is many times longer than that expected from simple diffusion of ATP out of the fiber, clearly supports the idea that it takes time for myosin heads to override tropomyosin to establish rigor state. The high Q_{10} value (~4) for the time of establishment of rigor state may be understood from the gradual spread of tropomyosin displacement, originating first at small regions within the fiber. Meanwhile, rigor tension decays rapidly to zero in ~1 s on returning the fiber to relaxing solution, being consistent with the calculation of ATP diffusion into the fiber [12].

Relation between rigor tension and rigor stiffness

In the present study, rigor tension and rigor stiffness were observed to increase in parallel with each other. As can be seen in Figure 2, however, the slope and the shape of tension versus stiffness curves were extremely variable from fiber to fiber. The variable rigor tension versus rigor stiffness curves seem to result from the complex action of myosin heads to override tropomyosin. When myosin heads mechanically displace tropomyosin around actin filaments to bind with actin, individual myosin heads would have to move taking various tension-generating configurations. The myosin head motion would cause local distortion of myofilament-lattice structures, which also produce additional tension. Rigor tension, recorded externally, is the sum of tensions caused by the motion of individual myosin heads to override tropomyosin, and therefore differ from fiber to fiber reflecting their myofilament-lattice organization.

On the other hand, muscle fiber stiffness, as measured by applying small length changes, may serve as a measure of the number of rigor linkages, as evidenced by the fact that, during isometric tension development of muscle fibers, development of muscle stiffness precedes that of tension [14,15]. On this basis, the extremely variable rigor tension versus rigor stiffness curves (Figure 2) can be accounted for as being due to variable conformation of individual myosin heads as well as variable distortion of myofilament-lattice. This idea seems to be consistent with the fact that, after reaching the peak value, rigor tension tends to start decreasing probably as the result of stress relaxation of distorted myofilament-lattice structures, while peak rigor stiffness remains unchanged (Figures 4-7). If the above explanation is correct, a large tension versus stiffness ratio indicates average tension per myosin head is large, while a small tension versus stiffness ratio results from small average tension per myosin head. The highly complex myofilament-lattice structures in low-Ca rigor fibers seems to be consistent with the report that the angle of spin labels attached to myosin heads in rigor fibers did not change by a static stress [16], since stretch may cause displacement of rigor myosin heads not only in the direction of stretch, but also in the direction opposite to that of stretch.

Mechanism of effect of antibodies to slow down development of rigor state

In the present study, antibodies 1, 2 and 3 markedly slowed down the rate of development of rigor tension and stiffness (Figures 4-7). A most plausible explanation for the effect of antibodies is that, the antibody molecules (IgG) should be detached from myosin heads before formation of rigor actin-myosin linkages. Since antibody 1 attaches to the distal region of myosin head CAD to cover actin-binding sites, we expected, at the start of present experiments, that antibody might strongly inhibit rigor linkage formation compared to the other antibodies. Unexpectedly, all the three antibodies were found to have a qualitatively similar effect to slow down the rate of development of rigor state. This result may be taken to indicate that, for rigor actin-myosin linkage formation, not only antibody 1 but also antibodies 2 and 3 should be detached from myosin heads by the mechanical action of myosin heads.

We have already shown that antibody 2, attaching to myosin head COD, has no effect on Ca^{2+}-activated muscle contraction and ATP-induced myosin head power and recovery strokes [7,17,18], but has marked inhibitory effect on ATP-dependent *in vitro* actin myosin sliding [7].

It follows from this that the action of myosin heads to override tropomyosin requires myosin head flexibility, which is necessary for *in vitro* actin-myosin sliding but not for muscle contraction [7], suggesting that myosin head movement in the formation of rigor actin-myosin linkages resembles that of *in vitro* actin-myosin sliding but not muscle contraction. Meanwhile, we have also shown that antibody 3, attaching to myosin head LD, has inhibitory action on muscle contraction but not on *in vitro* actin-myosin sliding, in which myosin head LD is mostly fixed on a glass surface [7]. This indicates that detachment of antibody 3 from myosin heads is necessary for their rigor linkage formation, being consistent with our previous reports that antibody 3 regulates binding strength of myosin heads to actin [19,20].

Conclusion

In the present study, we studied the effect of three different antibodies; antibody 1, 2 and 3, attaching to the distal region of myosin head CAD, myosin head COD, and myosin head LD, respectively, on the rate of development of rigor tension and stiffness in skinned vertebrate muscle fibers. Despite their different sites of attachment in myosin heads as well as their different effects on muscle contraction and *in vitro* actin-myosin sliding [7], their effect on the development of rigor state was qualitatively similar to one another; i.e. to slow down the rate of development of rigor state with or without changes in peak rigor tension and stiffness. The similarity in the effect of these antibodies may result from that myosin heads should override tropomyosin and detach antibodies to form rigor linkages with actin.

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References

1. Huxley HE, Hanson J (1954) Changes in the cross-striations of muscle during contraction and stretch and their structural impications. Nature 173: 973-976.
2. Lymn RW, Tayloe EW (1971) Mechanism of adenosine triphosphate hydrolysis by actomyosin. Biochemistry 16: 4617-4624.
3. Bagshaw CR (1993) Muscle contraction. Springer Science and Business Media, p: 155.
4. Geeves MA, Holmes KC (2005) The molecular mechanism of muscle contraction. Fibrous Proteins: Muscle and Molecular Motors 71: 161-193.
5. Sutoh K, Tokunaga M, Wakabayashi T (1989) Electron microscopic mappings of myosin head with site-directed antibodies. Journal of Molecular Biology 206: 357-363.
6. Minoda H, Okabe T, Inayoshi Y, Miyakawa T, Miyauchi Y, et al. (2011) Electron microscopic evidence for the myosin head lever arm mechanism in hydrated myosin filaments using the gas environmental chamber. Biochem Biophys Res Commun 405: 651-656.
7. Sugi H, Chaen S, Kobayashi T, Abe T, Kimura K, et al. (2014) Definite differences between in vitro actin-myosin sliding and muscle contraction as revealed using antibodies to myosin head. PLOS ONE 9: e93272.
8. Sugi H, Kobayashi T, Gross T, Noguchi K, Karr T, et al. (1992) Contraction characteristics and ATPas activity of skeletal muscle fibers in the presence of antibody to myosin subfragment 2. Proc Natl Acad Sci 89: 6134-6137.
9. Ebashi S, Endo M (1968) Calcium and muscle contraction. Progress in Biophysics and Molecular Biology 18: 123-183.
10. Kobayashi T, Kosuge S, Karr T, Sugi H (1998) Evidence for bidirectional functional communication between myosin subfragments 1 and 2 in skeletal muscle fibers. Biochem Biophys Res Commun 246: 539-542.
11. Squire JM (1981) The Structural Basis of Muscular Contraction, Springer, p: 716.
12. Sugi H, Yamaguchi M, Ohno T, Kobayashi T, Chaen S, et al. (2016) Tension recovery following following ramp-shaped release in high-Ca and Low-Ca rigor muscle fibers: Evidence for the dynamic state of AMADP myosin heads in the absence of ATP. PLOS ONE.
13. Kushmerick MJ, Podolsky RJ (1969) Ionic mobility in muscle cells. Science 166: 1297-1298.
14. Cecchi G, Griffiths PJ, Taylor S (1984) The kinetics of cross-bridge attachment and detachment studied by high frequency stiffness measurements. Contractile Mechanisms in Muscle, pp: 641-655.
15. Hatta I, Sugi H, Tamura Y (1988) Stiffness changes in frog skeletal muscle during contraction recorded using ultrasonic waves. J Physiol 403: 193-209.
16. Cooke R (1981) Stress does not alter conformation of a domain of the myosin cross-bridge I rigor muscle fibres. Nature 294: 570-571.
17. Sugi H, Minoda H, Inayoshi Y, Yumoto F, Miyakawa T, et al. (2008) Direct demonstration of the coss-bridge recovery stroke in muscle thick filaments in aqueous solution by using the hydration chamber. Proc Natl Acad Sci 105: 17396-17401.
18. Sugi H, Chaen S, Akimoto T, Minoda H, Miyakawa T, et al. (2015) Electron microscopic recording of myosin head power stroke in hydrated myosin filaments. Sci Rep 5: 15700.
19. Sugi H, Chaen S (2016) Evidence for the regulation of actin-myosin binding strength by lever arm and subfragment-2 regions of myosin molecule in contracting skinned muscle fibers as revealed by the effect of antibodies. J Nanomed Nanotechnol 7: 415.
20. Sugi H (2017) Evidence for the essential role of myosin subfragment-2 in muscle contraction: functional communication between myosin head and subfragment-2. J Mat Sci Eng 6: 386.