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Qualitatively different cross-bridge attachments in fast and slow muscle fiber types

Stefan Galler [a,*], Oleg Andruchov [a], Gabriela M.M. Stephenson [b], D. George Stephenson [c]

[a] Department of Cell Biology, University of Salzburg, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria
[b] Muscle Cell Biochemistry Laboratory, School of Biomedical and Health Sciences, Victoria University, P.O. Box 14428, MCMC, Melbourne, Vic. 8001, Australia
[c] Department of Zoology, La Trobe University, Vic. 3086, Australia

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ABSTRACT

Contractile properties differ between skeletal, cardiac and smooth muscles as well as between various skeletal muscle fiber types. This functional diversity is thought to be mainly related to different speeds of myosin head pulling cycles, with the molecular mechanism of force generation being essentially the same. In this study, force-generating attachments of myosin heads were investigated by applying small perturbations of myosin head pulling cycles in stepwise stretch experiments on skeletal muscle fibers of different type. Slow fibers (frog tonic and rat slow-twitch) exhibited only a 'slow-type' of myosin head attachment over the entire activation range, while fast fibers (frog and rat fast-twitch) displayed a 'slow-type' of myosin head attachment at low levels of activation, and an up to 30-times faster type at high levels of activation. These observations indicate that there are qualitative differences between the mechanisms of myosin head attachment in slow and fast vertebrate skeletal muscle fibers.

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Introduction

The force-generating attachment of myosin heads (cross-bridges) to the actin monomers on the thin filaments is primarily controlled by Ca^{2+} via the Ca^{2+}-regulatory system. Binding of Ca^{2+} to troponin C (TnC) causes a lateral shift of the inhibitory troponin complex and tropomyosin (TM) [1–3]. In this way, TM controls the binding of myosin heads to actin monomers. One TM molecule is thought to control a row of seven neighboring actins. To fully expose the binding sites and allow stereo-specific binding of myosin heads to actin with subsequent force generation, further displacement of TM is required, which exceeds that induced by Ca^{2+} binding to TnC [4,5]. For simplicity, the term "attachment" refers here to the binding of the myosin head to the actin filament with subsequent force generation.

For fulfilling different functional needs, fibers of skeletal, heart and smooth muscle as well as various skeletal muscle fiber types express different molecular species (isoforms) of myosin and regulatory proteins (for review, see [1,6]). Thus far it has been assumed that the molecular mechanism of force generation is essentially the same in all skeletal muscle fiber types. Since most investigations dealing with this subject were carried out under maximally activating conditions, we aimed to gain further insights into this process from experiments carried out at different levels of activation.

In the present study, force-generating attachments of myosin heads were investigated by applying small perturbations of myosin head pulling cycles in stepwise stretch experiments on single mechanically skinned skeletal muscle fibers. Rapid stretches of Ca^{2+}-activated fibers result in characteristic force transients which include a simultaneous rise in force with the stretch (phase 1), a rapid decay (phase 2) and a subsequent delayed force rise (phase 3) (Fig. 1A, top trace) [7–9]. The participation of myosin heads in these phases has been investigated in a number of previous studies [9–14]. The rapid force decay is thought to be due to shortening of the elastic element in cross-bridges following a change in their tilting angle and to the detachment of a small proportion of myosin heads. The delayed force rise (characterized by the time to peak, t_3, Fig. 1A, top trace) is thought to involve myosin head attachments. Former studies have shown that t_3 is tightly correlated with the myosin heavy chain (MHC) isoform composition of a fiber [8,15–17] indicating that t_3 can be used as a measure of attachment kinetics. The present measurements of t_3 at different levels of activation in fibers containing different MHC isoforms indicate a qualitative difference in cross-bridge attachment after stretch in fast and slow fibers.

Materials and methods

Frog iliofibularis (*Xenopus laevis*) and rat EDL muscles (Fisher 344) were quickly excised and single fibers were dissected under paraffin oil. Fibers were mechanically skinned by rolling back the

* Corresponding author. Fax: +43 662 8044 144.
E-mail address: Stefan.Galler@sbg.ac.at (S. Galler).

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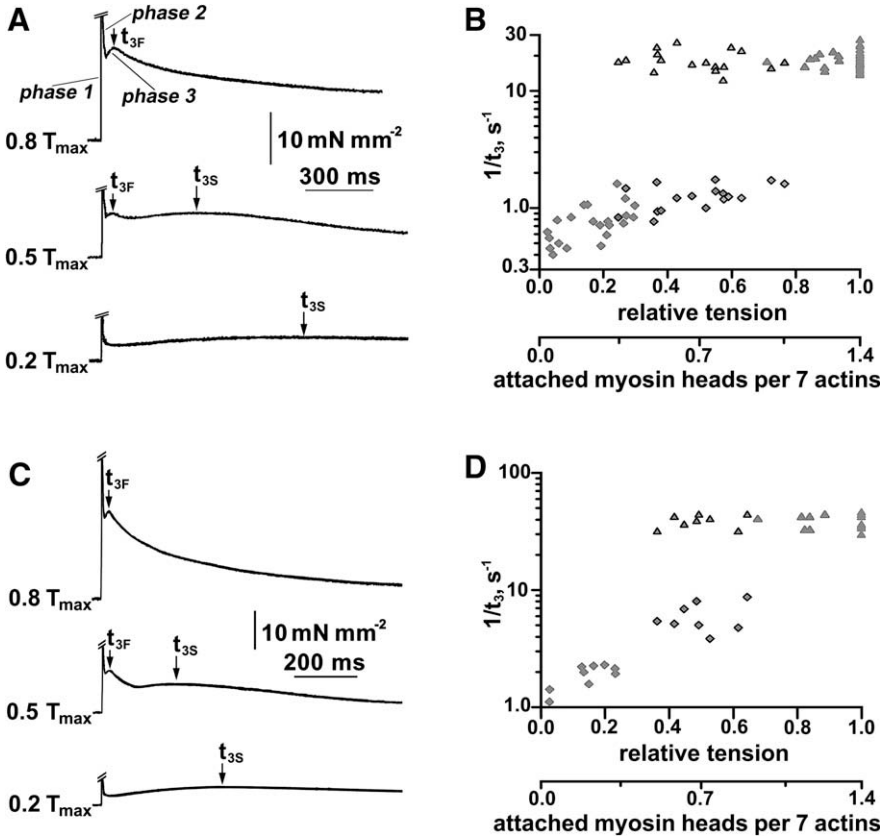

Fig. 1. Stretch–induced force transients of fast skeletal muscle fibers from frog (A) and rat (C) at different levels of activation. The tension traces start at the isometric value. The tension traces in A and C display a fast (t_{3F}) and/or a slow (t_{3S}) delayed force rise. The diagrams in B (frog fibers) and D (rat fibers) show the relationships between $1/t_{3S}$ and $1/t_{3F}$ values and the relative tension (expressed as a fraction of the maximal Ca^{2+}-activated tension) before the onset of the stretch. Rhombuses represent $1/t_{3S}$ values and the triangles represent $1/t_{3F}$ values. Dark edged symbols represent values from force transients containing both fast and slow delayed force rises. Results were obtained with 17 type X1 frog fibers (B) and 8 type IIB rat fibers (D). The second *X*-axis in B and D represents the calculated number of attached myosin heads per seven actins at different levels of activation. The calculation was based on measurements of stiffness, which in first approximation is proportional to the number of attached myosin heads. Rigor stiffness was used as a reference point because in rigor all myosin heads are attached to actin. In fast-twitch fibers the maximum number of myosin heads that can be attached per seven actins is 2. This value is based on data from X-ray diffraction studies of fast-twitch fibers (for review, see [28,29]) indicating 14 actins per 38 nm of actin filament length, 3 myosin heads per 14.3 nm myosin filament length and 2 actin filaments per one myosin filament ($\frac{7\times38}{14} \div \frac{14.3}{3} \div 2 = 1.99$). Stiffness at maximal Ca^{2+} activation was 70% of rigor stiffness suggesting that at maximal activation, 1.4 myosin heads are attached, on average, per seven actins. At lower levels of activation, the average number of attached myosin heads was considered to be proportionally less.

sarcolemma using fine forceps [18,19]. Fiber segments (1.4–2.4 mm) were mounted horizontally between two vertically orientated pins. One of the pins was the lever arm of a force transducer; the other pin was connected to a step motor, which allowed rapid changes of the fiber length (1–2 ms). Fibers were bathed in previously described solutions [18] based on (mM) 60 HEPES, 8 Na_2H_2ATP, 10 sodium creatine phosphate, 1 free Mg^{2+} and 50 mM EGTA. Free [Ca^{2+}], [Sr^{2+}] and [Mg^{2+}] were measured by ion-selective electrodes. After the mechanical experiments (frog and rat fibers at 15 and 22 °C, respectively), the fibers were collected for analysis of the MHC isoform content using SDS–PAGE [8,17].

Results and discussion

All fast-twitch fibers of frog and rat used in this study contained only MHC-X1 (pure X1 fibers) or MHC-IIb (pure IIB fibers), respectively. Likewise, all slow-twitch fibers of rat contained only MHC-I (pure type I fibers). Since the tonic isoform MHC-T has never been found to be expressed alone in frog fibers [17], we used X1-T fibers expressing >60% MHC-T and <40% MHC-X1. Henceforth, these tonic fibers of frog and slow-twitch fibers of rat are called "slow fibers"; while fast-twitch fibers of frog and rat are called "fast fibers".

Original traces of stretch–induced force transients of single frog and rat fibers, measured at different levels of Ca^{2+} activation, are shown in Fig. 1 (fast fibers) and Fig. 2 (slow fibers). At activation levels >0.75 of maximum force (T_{max}), the stretch–induced delayed force rise peaked at about 50–70 ms and 20–30 ms in fast fibers of frog and rat, respectively (Fig. 1A and C). In contrast, the stretch–induced delayed force rise peaked at >2.8 s and >0.9 s in slow fibers of frog and rat, respectively (Fig. 2A and C). With decreasing activation, the stretch–induced delayed force rise slowed down gradually in slow fibers (Fig. 2A–D). This is similar to what was reported for mouse cardiac muscle [14]. In contrast, in fast fibers at activation levels between about 0.75 and 0.25 T_{max} (Fig. 1A and C), force traces typically exhibited not one but two types of stretch–induced delayed force rises: a fast-type (t_{3F}), seen also at higher levels of activation, and in addition, a much slower type (t_{3S}) which was not dissimilar from that observed in the slow fibers (Fig. 2A and C). At activation levels <0.25 T_{max} only the slow-type was observed. As seen in Fig. 1B and D, the $1/t_{3F}$ values are fairly independent of force level, whereas the $1/t_{3S}$ values, like those for slow fibers, increase continuously with the increase in force. These results indicate that in fast fibers there are two qualitatively different types of myosin head attachment, a slow-type of attachment and a fast-type of attachment.

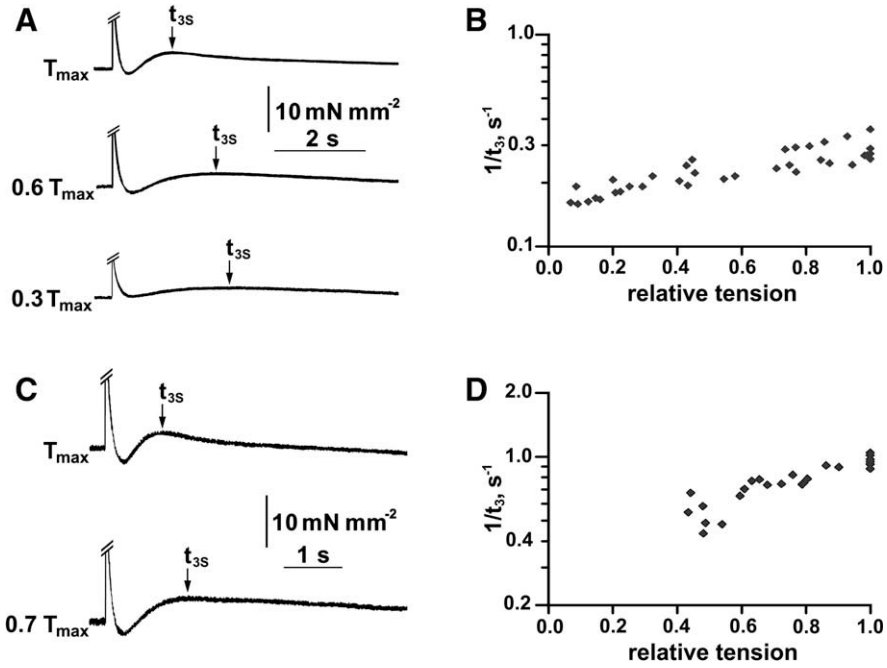

Fig. 2. Stretch–induced force transients of slow skeletal muscle fibers from frog (A) and rat (C) at different levels of activation. Results were obtained with 4 type X1-T slow-tonic frog fibers (B) and 10 slow-twitch type I rat fibers (D). For details see legend of Fig. 1.

With fast fibers, further experiments were executed in which the activation level was changed by varying the sarcomere length (SL) (Fig. 3A and B) or free [Mg^{2+}] (Fig. 3C and D) at constant submaximally activating [Ca^{2+}] or by using Sr^{2+} instead of Ca^{2+} (Fig. 3E and F). These experiments demonstrate that the two types of attachment in fast fibers depend on the activation level (i.e. the fraction of attached myosin heads) and not on the level of Ca^{2+}.

The gradual increase of slow-type attachment kinetics with the level of activation (in fast and slow fibers as well as in cardiac muscle [14]) can be explained by considering that adjacent TM molecules along the actin filament are connected end to end [20] and interact cooperatively. In such case, the shift of one TM molecule promotes a partial shift of the neighboring TM molecules. As a result, the attachment of myosin heads in the region controlled by partially shifted TM molecules is expected to be faster. The sudden appearance of the fast-type of attachment in fast fibers at intermediate and high levels of activation must involve an additional mechanism from that described above. This mechanism must depend in some way on the density of attached myosin heads, which increases proportionally with the level of activation. The density of attached myosin heads per row of seven actins is shown on a second abscissa of Fig. 1B and D. This was calculated based on the known molecular structure of the myosin and actin filaments and on stiffness measurements (see legend of Fig. 1). At low activation levels, when fewer than 0.35 myosin heads are attached on average per row of seven actins (one head attached for a row of more than about 20 actins), the attachment is always slow (Fig. 1A–D). Under these conditions it is unlikely to have rows of seven actins with more than one myosin head attached. Therefore, when myosin heads are perturbed by the rapid stretch they will necessarily attach to rows of seven actins that do not have another myosin head already attached. Consequently, these attachments will be relatively slow because TM must be shifted to the fully displaced position to uncover the myosin head binding site on actin. In contrast, at high levels of activation, when more than one myosin head is attached per row of seven actins, the attachment will be always fast, as the attached myosin heads keep the TMs in the fully displaced position. Thus, the fast-type of attachment seems to involve a facilitation mechanism whereby myosin heads already attached to actin substantially accelerate the further attachment of neighboring myosin heads. At intermediate levels of activation, the attachment of particular myosin heads will be either slow or fast depending on whether the corresponding myosin binding sites on actin are partially covered by TM, or are fully uncovered. Another possibility for explaining the two types of attachment is to assume that the slow-type of attachment involves only one head of the double headed myosin, whereas the fast-type of attachment involves both heads [21]. Other explanations based on plausible differences in TM position shifts in slow and fast fibers are also possible. The existence of two qualitatively different attachments in fast and slow fibers is supported by structural studies showing that myosin heads of fast and slow muscles execute different conformational changes upon ADP binding [22].

The sudden change from the slow- to the fast-type of attachment in fast fibers is physiologically important because it allows a rapid change in the rate of force development at high levels of activation. In contrast, the presence of only slow-type of attachment, with moderate dependence on level of activation, enables slow fibers and cardiac muscle cells to become faster gradually at higher levels of activation. It has been known for a long time that force develops (and redevelops after a period of isotonic release) faster at higher Ca^{2+} levels, and this is much more pronounced in fast than in slow fibers [18,23–26] or cardiac muscle [27]. These features can be fully explained by the different myosin head attachment mechanisms that operate in fast and slow skeletal muscle fibers and in cardiac muscle.

In summary, our results provide new insights into functional aspects of muscle diversity by showing qualitative differences in cross-bridge attachment of myosin heads after stretch in slow and fast vertebrate skeletal muscle fibers over a wide range of activation. Further investigations using different experimental approaches are required to determine the underlying molecular mechanisms.

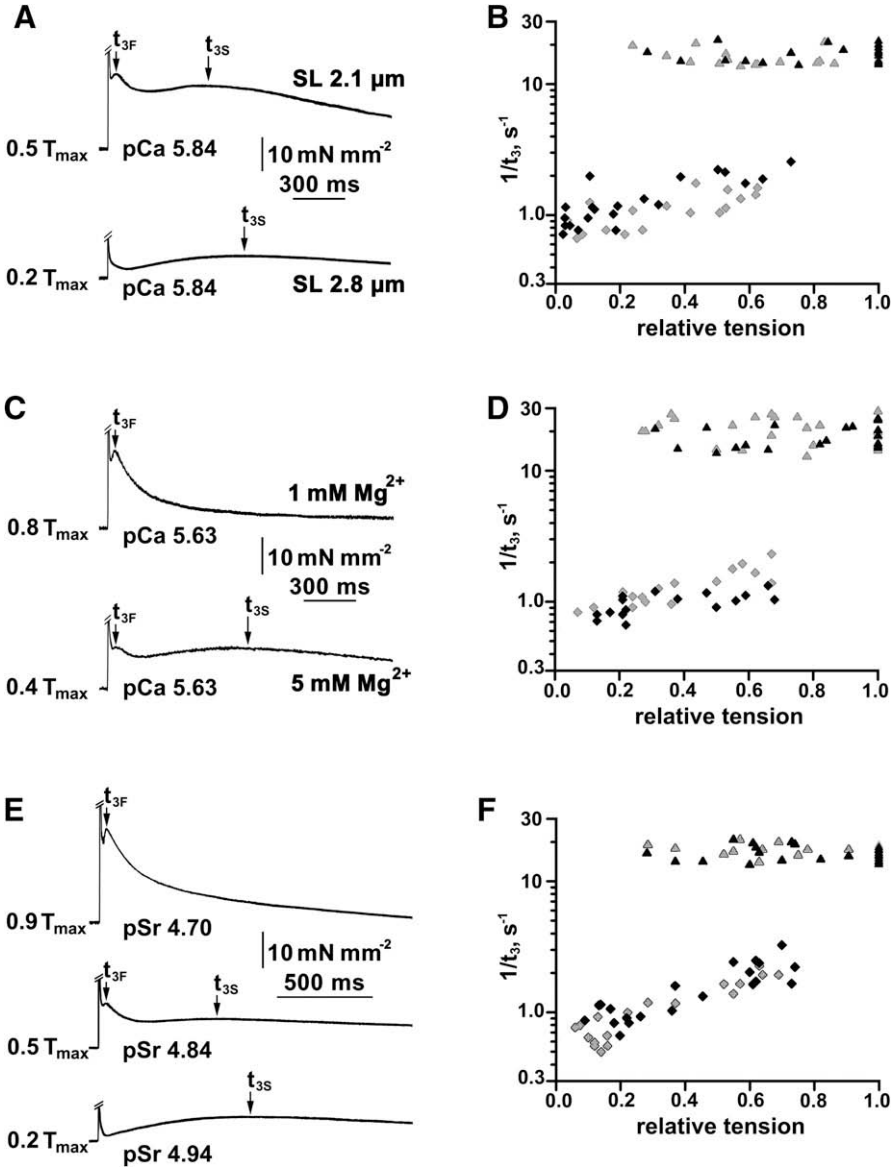

Fig. 3. Stretch–induced force transients of fast-twitch frog type X1 fibers measured under different experimental conditions. Fibers were activated submaximally at constant [Ca^{2+}] and different SLs (A), at constant [Ca^{2+}] and different free [Mg^{2+}] (C), or at different [Sr^{2+}] expressed as pSr (E). The diagrams in B, D, and F show corresponding relationships between $1/t_{3F}$ (triangles) and $1/t_{3S}$ (rhombuses) values and relative force. The light grey symbols in B, D, and F represent values measured on 12, 7, and 10 fibers, respectively, under standard conditions. The dark grey symbols represent values measured in the same respective fibers when one of the standard parameters was altered (B, 2.1 µm SL; D, 5 mM [Mg^{2+}]; F, activation by Sr^{2+} instead of Ca^{2+}). Note that the force decrease induced by changes in SL or [Mg^{2+}] caused a transition from the fast to the slow delayed force rise (A and C). Activation by Sr^{2+} produced similar results to those produced by Ca^{2+} (E and F).

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References

[1] J.C. Rüegg, Calcium in Muscle Contraction, Springer, Berlin Heidelberg, 1992.
[2] P. Vibert, R. Craig, W. Lehman, Steric model for activation of muscle thin filament, J. Mol. Biol. 266 (1997) 8–14.
[3] A.M. Gordon, E. Homsher, M. Regnier, Regulation of contraction in striated muscle, Physiol. Rev. 80 (2000) 853–924.
[4] D.F.A. McKillop, M.A. Geeves, Regulation of the interaction between actin and myosin subfragment 1: evidence for three states of the thin filament, Biophys. J. 65 (1993) 693–701.
[5] S.S. Lehrer, N.L. Golitsina, M.A. Geeves, Actin-tropomyosin activation of myosin subfragment 1 ATPase and thin filament cooperativity. The role of tropomyosin flexibility and end-to-end interactions, Biochemistry 36 (1997) 13449–13454.
[6] S. Schiaffino, C. Reggiani, Molecular diversity of myofibrillar proteins: gene regulation and functional significance, Physiol. Rev. 76 (1996) 371–423.
[7] R.H. Abbott, G.J. Steiger, Temperature and amplitude dependence of tension transients in glycerinated skeletal and insect fibrillar muscle, J. Physiol. 266 (1977) 13–42.
[8] S. Galler, T. Schmitt, D. Pette, Stretch activation, unloaded shortening velocity, and myosin heavy chain isoforms of rat skeletal muscle fibres, J. Physiol. 478 (1994) 513–521.
[9] M. Linari, M.K. Reedy, M.C. Reedy, V. Lombardi, G. Piazzasi, Ca^{2+}-activation and stretch-activation in insect flight muscle, Biophys. J. 87 (2004) 1101–1111.
[10] A.F. Huxley, R.M. Simmons, Proposed mechanism of force generation in striated muscle, Nature 233 (1971) 533–538.
[11] A.F. Huxley, R.M. Simmons, Mechanical transients and the origin of muscular force, Cold Spring Harbor Symp. Quant. Biol. 37 (1973) 669–680.
[12] L.E. Ford, A.F. Huxley, R.M. Simmons, Tension responses to sudden length change in stimulated frog muscle fibres near slack length, J. Physiol. 269 (1977) 441–515.

[13] A.F. Huxley, Cross-bridge action: present views, prospects, and unknowns, J. Biomech. 33 (2000) 1189–1195.
[14] J.E. Stelzer, L. Larsson, D.P. Fitzsimons, R.L. Moss, Activation dependence of stretch activation in mouse skinned myocardium: implication for ventricular function, J. Gen. Physiol. 127 (2000) 95–107.
[15] S. Galler, K. Hilber, D. Pette, Force responses following stepwise length changes of rat skeletal muscle fibre types, J. Physiol. 493 (1996) 219–227.
[16] O. Andruchov, O. Anduchova, Y. Wang, S. Galler, Kinetic properties of myosin heavy chain isoforms in mouse skeletal muscle: comparison with rat, rabbit and human and correlation with amino acid sequence, Am. J. Physiol. 287 (2004) C1725–C1732.
[17] O. Andruchova, G.M. Stephenson, O. Andruchov, D.G. Stephenson, S. Galler, Myosin heavy chain isoform composition and stretch activation kinetics in single fibres of *Xenopus laevis* iliofibularis muscle, J. Physiol. 574 (2006) 307–317.
[18] D.G. Stephenson, D.A. Williams, Calcium activated force responses in fast- and slow-twitch skinned muscle fibres of the rat at different temperatures, J. Physiol. 317 (1981) 281–302.
[19] S. Galler, K. Hilber, Unloaded shortening of skinned mammalian skeletal muscle fibres. Effects of the experimental approach and passive force, J. Muscle Res. Cell Motil. 15 (1994) 400–412.
[20] R.D. Bremel, A. Weber, Cooperation within actin filament in vertebrate skeletal muscle, Nature 238 (1972) 97–101.
[21] E. Brunello, M. Reconditi, R. Elangovan, M. Linari, Y.B. Sun, T. Narayanan, P. Panine, G. Piazzesi, M. Irving, V. Lombardi, Skeletal muscle resists stretch by rapid binding of the second motor domain of myosin to actin, Proc. Natl. Acad. Sci. USA 104 (2007) 20114–20119.
[22] H. Iwamoto, K. Oiwa, M. Kovacs, J.R. Sellers, T. Suzuki, J. Wakayama, et al., Diversity of structural behavior in vertebrate conventional myosins complexed with actin, J. Mol. Biol. 369 (2007) 249–264.
[23] D.G. Moisescu, Kinetics of reaction in calcium-activated skinned muscle fibres, Nature 262 (1976) 610–613.
[24] B. Brenner, Effect of Ca^{2+} on cross-bridge turnover kinetics in skinned single rabbit psoas fibers: implications for regulation of muscle contraction, Proc. Natl. Acad. Sci. USA 85 (1988) 3265–3269.
[25] J.M. Metzger, R.L. Moss, Calcium-sensitive cross-bridge transitions in mammalian fast and slow skeletal muscle fibers, Science 247 (1990) 1088–1090.
[26] K. Campbell, Tension recovery in permeabilized rat soleus muscle fibers after rapid shortening, restretch, J. Biophys. 90 (2006) 1288–1294.
[27] M.R. Wolff, K.S. McDonald, R.L. Moss, Rate of tension development in cardiac muscle varies with level of activator calcium, Circ. Res. 76 (1995) 154–160.
[28] L.S. Tobacman, Thin filament-mediated regulation of cardiac contraction, Annu. Rev. Physiol. 58 (1996) 447–481.
[29] R. Craig, J.L. Woodhead, Structure and function of myosin filaments, Curr. Opin. Struct. Biol. 16 (2006) 204–212.