CALCIUM AND TENSION-DEPENDENT CHANGES IN THE ACTIN FILAMENT STRUCTURE OF INSECT FIBRILLAR MUSCLE

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1. Introduction

One tropomyosin or *TM* chain lies in each of the two grooves of the actin helix, with one 40 nm long TM rod interacting with seven actin monomers [1]. The TM can move between two positions, the *relaxed* state where it is situated close to the edge of the actin groove and the *activated* position with the TM near the center of the groove [2–4]. As the contact area between the myosin head and the actin extends round into the groove [5] the relaxed position may ensure steric blocking of the myosin attachment [3]. Under relaxing conditions the troponin complex at 38.5 nm intervals may hold the TM in the inhibitory position until Ca^{2+} becomes available [3]. Experiments on rigor muscle have shown that attaching myosin cross-bridges are able to move the TM towards the center of the groove [2], which may increase the probability of additional cross-bridge attachment [6].

As in earlier work [2–4] the changes on the second actin layer-line at 19.3 nm have been used as a measure for the movement of the TM.

In glycerinated insect muscle the intensity of the 19.3 nm reflection increases in proportion both with the Ca^{2+} level and with the tension load on the fibres. At 10^{-5} M Ca^{2+} the 19.3 nm increase exhibits a slight phase advance on tension. Further, most bridges on the 14.5 nm myosin lattice changed their angle 10–20 msec later than was true for the maximum of the 19.3 nm increase and by implication for the tropomyosin movement.

2. Material and methods

Bundles of 20 freshly glycerinated fibres from the back muscle of the water bug *Lethocerus colossicus* have been used. The experimental conditions and the mechanical apparatus (which can be operated both under length and tension clamp) have been described previously [7].

The composition of the immersion media was the same as given by White and Thorson [8]. At 10^{-9} M Ca^{2+} in the relaxing solution the fibres developed a small tension when stretched by 2% above rest length. Similar stretches of 2% were also set up in the activating solution [8]. The tension developed at this length in presence of either 10^{-7} M or 10^{-5} M Ca^{2+} was then reduced by the apparatus in successive steps of 20–60%. In a second type of experiment the fibres were sinusoidally oscillated. The rigor state could be induced by immersing the fibre bundle in an ATP-free solution [7, 8]. As an *X*-ray source white radiation from a Synchrotron (Institute of Nuclear Physics, Novosibirsk; operated in storage mode at 0.91 GeV, 140 mA beam current) was employed. The quartz monochromator and the design of the camera was similar to that of Rosenbaum et al. [9]. The distance between Synchrotron and crystal was 17 m. As the electron pulses were emitted at 50 Hz frequency the phase relation with respect to the oscillating muscle was varied so that the *X*-radiation was emitted in turn at 20, 40, 60, 80, 100 msec and at 10, 30, 50, 70 and 90 msec during the 100 msec oscillation cycle to get a well-averaged energy at each point sampled. The line focus was foreshortened to a spot of 220 μm × 260 μm, giving a flux of 2×10^{11} photons/sec at the chosen wavelength of 1.5 Å. A lead mask was placed in the focal plane, the two apertures (selected in turn) allowed a recording of the 19.3 nm layer-line sampled by the 1,0 row line

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and during sinusoidal oscillation also of the intensity of the 14.5 nm meriodonal spot [10]. As an internal standard the first equatorial layer-line (E_{1R}) was recorded.

3. Results

For glycerinated fibres under relaxing and rigor conditions the intensities of the 19.3 nm reflection were 0.08% E_{1R} and 0.3% E_{1R}, respectively, corresponding to average counting rates of 6.7 cps and 25.1 cps. The passive tensions developed were 1.8 mg/fibre in the relaxed state at 10^{-9} M Ca^{2+} and 10.5 mg/fibre in rigor muscle.

Increasing the Ca^{2+} level from 10^{-9} M to 10^{-5} M in a muscle held isometrically at 2% stretch virtually doubles the 19.3 nm intensity. To investigate the effect of tension, which is known to be responsible for ATPase activation [11, 12] the tension developed by the fibres was reduced to 20–60% by the apparatus. The limited amounts of fibre shortening which accompany the step reductions in tension load (table 1) are too small to affect the filament overlap.

As becomes apparent from the data in table 1 tension has as much an effect on the intensity increase as the Ca^{2+} level in the medium. Actually, when at 10^{-5} M Ca^{2+} the tension is reduced to 1.5 mg/fibre the intensity is now close to that at 10^{-7} M Ca^{2+} and a tension load of 6.25 mg/fibre. For comparable amounts of tension at both Ca^{2+} concentrations the intensity difference is almost constant.

Table 1
Changes in the 19.3 nm intensity in relation to Ca^{2+} concentration and tension development

Ca^{2+} level	Tension loading	% Shortening	Intensity at 19.3 nm
10^{-7} M	6.25 mg/fibre	–	0.102 ± 0.008 (6)
10^{-7} M	3.75 mg/fibre	0.75	0.094 ± 0.011 (5)
10^{-7} M	1.25 mg/fibre	1.40	0.086 ± 0.009 (5)
10^{-5} M	7.50 mg/fibre	–	0.164 ± 0.014 (6)
10^{-5} M	6.25 mg/fibre	0.27	0.154 ± 0.016 (5)
10^{-5} M	3.75 mg/fibre	0.80	0.141 ± 0.015 (4)
10^{-5} M	1.50 mg/fibre	1.65	0.114 ± 0.009 (5)

The intensity changes are given as mean ± S.E. (no. of experiments).

To test the involvement of tropomyosin movem in the dynamic activation the fibres were oscillatec 10^{-5} M Ca^{2+} with a power-producing frequency o 10Hz. Each of the fibres performed 0.6 ncal of wo per oscillation cycle at a peak-to-peak tension of 9.4 mg. As can be seen from fig. 1 the 19.3 nm intensity attains a maximum half-way through the oscillation cycle, preceding even slightly the tensio maximum. Actually, with 0.36% E_{1R} the 19.3 nm maximum was higher during oscillatory contractio than in the rigor state, for which it remained at 0.3 E_{1R} both under static and dynamic conditions. In comparison, Armitage et al. [13], using a lower oscillation frequency, observed a smaller 19.3 nm increase almost in phase with tension.

As shown in fig. 1 the reduction in the oscillato tension and the decrease in 19.3 nm intensity virtually coincide.

To test the hypothesis that the movement of th tropomyosin is coupled in some way to the move- ment of the myosin cross-bridges use was made of finding that the intensity of the 14.5 nm reflectio is related to the number of cross-bridges on the 14.5 nm myosin lattice and to the angle which suc cross-bridges make with the myosin filament axis [10]; an intensity decrease is proportional to myo bridges moving axially. However, when the change on the 14.5 nm repeat were recorded in parallel to the 19.3 nm reflection, it can be demonstrated (fi that at 10 Hz there are still relatively few bridges leaving the relaxed myosin lattice when the 19.3 n intensity is already maximal. Further, it may be important for the kinetics of the cross-bridge cycl that the over-all angle change of the cross-bridge population actually lags behind the tension develo ment by about 10 msec.

4. Discussion

The present findings on insect fibrillar muscle a consistent with the model predictions advanced fo vertebrate striated muscle [2–4]. The observation that in rigor the 19.3 nm intensity is 3.75 times th in the relaxed state can be reconciled with a small change in the angle between the tropomyosin and the actin helices.

In insect muscle even maximally activating Ca^{2+}

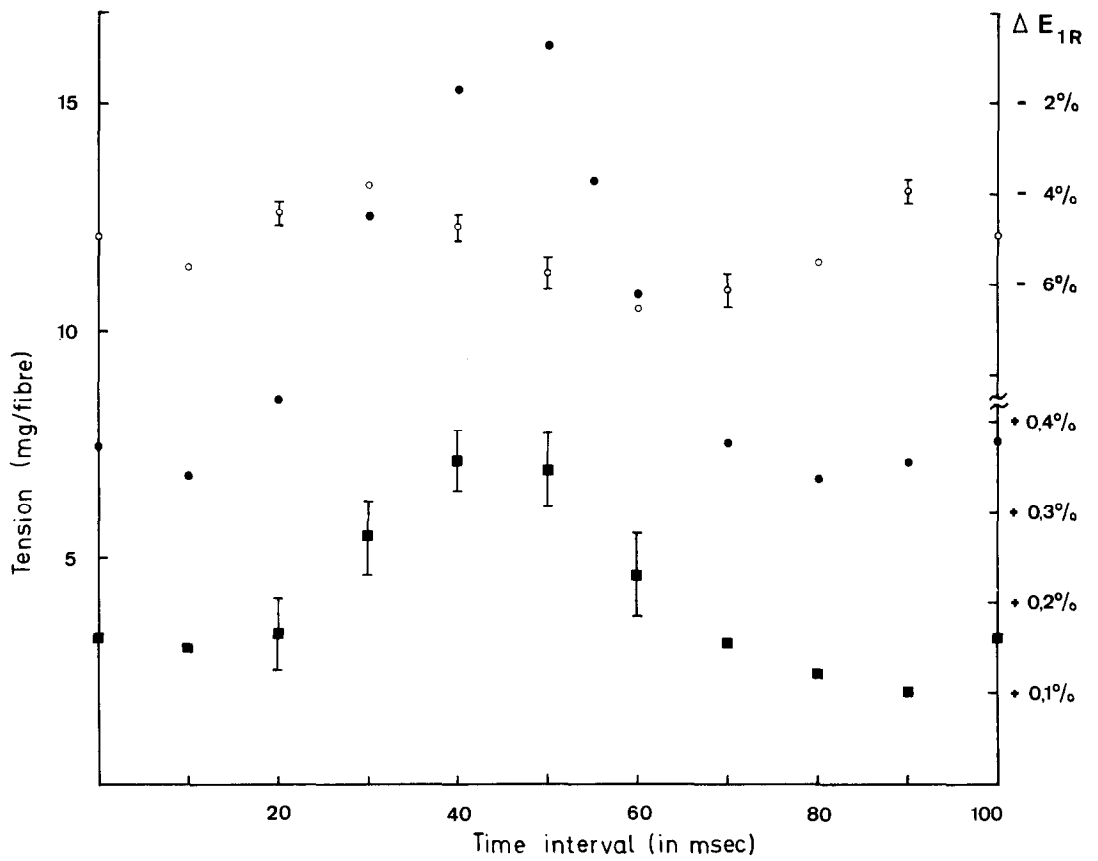

Fig. 1. The phase relation between intensity changes at 19.3 nm and 14.5 nm and tension development in an oscillatory contraction–relaxation cycle. In presence of 10^{-5} M Ca^{2+} the fibres were sinusoidally oscillated at a length amplitude of 50 μm or 1% peak-to-peak and a frequency of 10 Hz. The intensity changes of the 14.5 nm reflection (o) and at 19.3 nm (▪), as deduced from the counts sampled at 10 msec intervals, have been expressed as percentage changes of the first equatorial reflection (E_{1R}). The corresponding tension changes have been indicated by the solid circles (•). Each of the points represents the mean of 15 experiments with the standard error indicated by the cross bars.

levels of 10^{-5} M fail to induce maximal intensity changes at 19.3 nm. The amount of tension on insect muscle constitutes an additional factor which controls the intensity change, although improved orientation may make an unknown contribution. It is quite possible that an excess of inhibitory factor in the insect troponin complex [14] places a more stringent bonding on the tropomyosin, which can only be released through a mechanical effect exerted by the attaching cross-bridges. Such a mechanism would equally explain the observed increase in calcium binding with stretch and hence tension [11], with the attaching bridges inducing a high affinity Ca-binding state of the troponin in the way postulated by Bremel and Weber [6].

The phase relation between the intensity changes at 19.3 nm and at 14.5 nm and the oscillatory tension development suggests that it may be attachment of the myosin bridges and not their subsequent axial movement which leads to tropomyosin displacement.

In order to explain the finding of the 19.3 nm intensity being higher during oscillatory contraction–relaxation cycles than under rigor conditions one should bear in mind that in insect fibrillar muscle the 19.3 nm reflection constitutes a second-order layer-line not only for the actin but also for the myosin helix [10, 13].

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