CHANGES IN MYOSIN CROSS-BRIDGE ATTACHMENT DURING OSCILLATORY CONTRACTIONS OF INSECT FIBRILLAR MUSCLE

R.A. CHAPLAIN and B. HONKA

Department of Biocybernetics, Advanced College of Technology, Magdeburg, Kleiberweg 14, GDR

Received 15 January 1974

1. Introduction

The results of low-angle X-ray diffraction studies on glycerinated insect fibrillar muscle fit an actin helix of pitch 5.92 mm with an axial subunit repeat of 2.75 nm. The strongest actin layer-line is an off-meriodonal reflection at 5.9 nm [1, 2]. In the relaxed state all the intensity is distributed parallel to the equator, while on going into rigor the 5.9 nm intensity increases and the density is now streaking across the meridian [1]. As there are no changes in the 5.9 nm reflection between the relaxed and the rigor state, the actin helix appears to remain unchanged [1, 3, 4].

Based on detailed model calculations the increase in intensity and the changed density distribution in the rigor state has been attributed to the extra mass of the myosin cross-bridge heads which become stably attached to the actin monomers [1, 5].

In the present study the changes have been investigated at 5.9 nm for muscles undergoing oscillatory contraction–relaxation cycles. The intensity changes have been recorded only for the near-meriodonal part along the 5.9 nm layer-line which is most sensitive to cross-bridge attachment [5]. At power-producing frequencies of 1 Hz, 5 Hz, 15 Hz and 20 Hz information has been obtained on the phase relation between active tension development and fluctuations in the mass of the myosin heads which attach to the actin at a given angle.

The basic outcome of these studies was that the 5.9 nm intensity is initially decreasing during the sinusoidal stretching phase and always increasing towards the end of the oscillatory cycle. Further, the sampled 5.9 nm intensity reaches a maximum halfway through the oscillation cycle which only at 1 Hz and 20 Hz coincides with the tension maximum but at 5 and 155 Hz is lagging behind the tension peak.

2. Materials and methods

Bundles of 20 glycerol-extracted fibres from the dorsal longitudinal muscle of the water bug *Lethocerus colossicus* (stored at −18°C for 4–8 days in 50% glycerol, pH 6.9) were glued to a pair of glass rods, one connected to a strain gauge (WWH 141) tension transducer, the other to a vibrator [6]. The central portion of the fibre bundle was sandwiched between two Mylar windows to allow the X-ray beam to pass through A Huxley–Holmes-type rotating anode X-ray tube was used (loading 35 kV), giving a foreshortened spot of 150 μm × 180 μm. Of the density distributed along the 5.9 nm layer-line only the density near the meridian up to an equatorial spacing of 0.05 nm^{-1} was sampled by a proportional counter. The lead mask placed in the focal plane was proportioned to take in two quadrants so as to increase the sensitivity. The counts were accumulated in the relaxed and rigor states or at a given interval in the oscillatory cycle for a standard period of 30 min, thus providing a constant background. As an internal standard the first equatorial reflection was measured by selecting in turn a second aperture of the metal mask.

In the experiments the fibre bundle was initially immersed in relaxing solution (15 mM Na_2-ATP, 7 mM $MgCl_2$, 5 mM Na-azide, 4 mM EGTA, 20 mM K-phosphate buffer, pH 6.8; the ionic strength was adjusted to 0.12 with KCl) and the fibre length there-

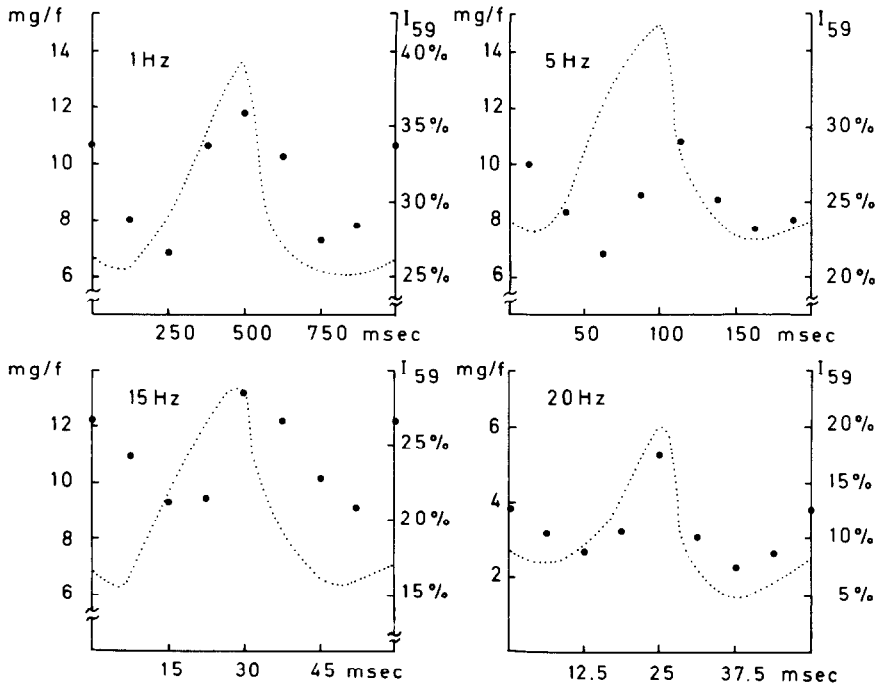

Fig. 1. Tension development and changes in the near-meriodonal density distribution on the 5.9 nm layer-line. The respective oscillation frequencies are given in the diagram. The tension changes indicated by the dotted line were monitored continuously. The aggregate of counts accumulated on the 5.9 nm layer-line at time intervals equal to every eighth of the oscillatory cycle are represented by the solid circles. All data represent the least square fit to 15 experiments obtained by a computer. The standard error of the 5.9 nm intensity changes varied between 8–12%.

after extended by 2% above the length which gave zero tension. After the X-ray recording the fibres were transferred to the activating solution of 10^{-5} M Ca^{2+}, stabilized with Ca-EGTA [6]. When ATP was absent from the otherwise identical composition of the relaxing solution the fibres went into rigor [7].

3. Results

A suitable set of reference values was obtained by recording the 5.9 nm intensity for each bundle in the relaxed and rigor state. To be able to express these changes in absolute terms they are best related to the intensity recorded for the first equatorial layer-line (E_{1R}) which gave highly reproducible values.

The sampled 5.9 nm intensity was 0.91 ± 0.06% E_{1R} (mean ± S.E., n = 15) under relaxing conditions where the cross-bridges are detached [7]; in the rigor state it amounted to 1.88 ± 0.16% E_{1R} (mean ± S.E., n = 15).

For the quantitative evaluation of the data on active muscle the 5.9 nm intensity in the relaxed state was always subtracted and the difference to the rigor state defined as unity. The intensities sampled during each eighth of the oscillatory cycle were then expressed as a percentage of this difference, I_{59} being 100% for $I_{rigor} - I_{relaxed}$.

In the sinusoidal stretch-and-release cycles imposed on the fibres at a peak-to-peak amplitude of 1% or 50 μm and the frequencies given in fig. 1, the length changes were always followed by delayed tension changes, with the fibres performing oscillatory work. The time intervals for measuring the X-ray intensity were deliberately chosen so as for one of them to coincide with the time of the maximal intensity increase at the particular frequency.

The density changes on the 5.9 nm layer-line in the

course of an oscillatory length-tension cycle reveal a definite phase relationship which is characteristic for the oscillation frequency (fig. 1). In absolute terms the greatest intensity increase is observed at a frequency of 1 Hz, being lowest at 20 Hz.

Although the magnitude of the peak-to-peak tension change is comparable at 1 Hz, 5 Hz and 15 Hz, the 5.9 nm intensity fluctuates between 27–36% at 1 Hz, 22–29% at 5 Hz, and between 21–28% at 15 Hz (fig. 1).

The lack of correlation between the sampled near-meriodonal density at 5.9 nm and the amount of tension developed becomes more striking still, if one considers the phase relation over the whole oscillation cycle. Only at 1 Hz and 20 Hz did the maxima of the intensity and of the tension coincide, while at 5 and 15 Hz the intensity attains its maximum later.

At the end of the release phase and hence on the starting point for the initiation of the next oscillatory cycle the intensity was always high, but becomes reduced half-way through the stretching phase. After the 5.9 nm intensity has reached its peak value at a time when the muscle is already reduced below its mean length the intensity drops again, although the relative extent of this reduction in intensity is much less than the accompanying decrease in tension (see particularly 1 Hz and 15 Hz). At a frequency of 20 Hz both the oscillatory tension and the density values are reduced. However, there is a peak-to-peak change of 10% in intensity (7.2–17.5%) for a peak-to-peak change of 4.5 mg/fibre, while for a peak-to-peak tension amplitude of 7 mg/fibre at 1–15 Hz it varied between 9% and 7%. Certainly, not too much significance can be attached to small intensity differences of 1–2% in view of a standard error of about 10%. In fact a 2% change in I_{59} corresponds to a mean aggregate of 762 counts per 30 min and that on a background of about 34 600 counts.

4. Discussion

The intensity changes on the 5.9 nm layer-line close to the meridian, although certainly related to cross-bridge attachment to the actin filament [1, 5], exhibit no simple relation to the magnitude or the time course of the oscillatory tension changes (fig. 1). This seems not altogether surprising as a proportionality between the number of actin–myosin links and the 5.9 nm intensity increase requires that all these cross-bridges are attached to the actin at an almost constant angle [1, 5]. Particularly the intensity increase at the end of the oscillation cycle which is unaccompanied by a major tension development will be difficult to explain on the basis of a constant angle of attachment inherent in the modelling of the rigor data [1, 5].

To explain the kinetics of the density changes shown in fig. 1 the following working hypothesis is offered which utilizes the predictions and calculations of previous models [1, 5]. As the result of stretch still existing actin–myosin links dissociate; in the second half of the stretching phase new cross-bridges attach to the actin and generate tension, with the angle between myosin head and actin being 35°C both axially and azimuthally and hence the intensity high [1]. When the fibre length is reduced at increasing speed the cross-bridge head may be moved axially to an angle of 45°C or more which would lower the intensity close to the meridian. To account for the dip in intensity after the tension maximum it could additionally be envisaged that during the sinusoidal release phase the cross-bridge head shortens from the normal banana-shaped appearance [8] (assumed to be 11.2 nm in length and 5.6 nm in diameter in insect muscle [1]) to an egg-like conformation as has been observed in hydrodynamic studies [9], where both the length and the diameter of the head was only 7 nm. The density increase at the end of the oscillation cycle can be explained by postulating that the axial tilt of the myosin head becomes much less as the muscle re-approaches its mean length, possibly with the myosin head still in the shorter conformation. The imposed movement of 6 nm per half-sarcomere would certainly account for the required decrease in the cross-bridge angle.

References

[1] Miller, A. and Tregear, R.T. (1972) J. Mol. Biol. 70, 85–104.
[2] Miller, A. and Tregear, R.T. (1971) in: Symposium on Contractility (Podolsky, R.J., ed.), pp. 205–228, Prentice Hall.
[3] Huxley, H.E. and Brown, W. (1967) J. Mol. Biol. 30, 383–395.
[4] Armitage, P., Miller, A., Rodger, C.D. and Tregear, R.T. (1972) Cold Spring Harbor Symp. Quant. Biol. 37, 379–387.

[5] Parry, D.A.D. and Squire, J.M. (1973) J. Mol. Biol. 75, 33–55.
[6] Abbott, R.T. and Chaplain, R.A. (1966) J. Cell. Sci. 1, 311–324.
[7] Reedy, M.K., Holmes, K.C. and Tregear, R.T. (1965) Nature 207, 1276–1281.
[8] Moore, P.B., Huxley, H.E. and DeRosier, D. (1970) J. Mol. Biol. 50, 279–296.
[9] Lowey, S., Slayter, H., Weeds, A. and Baker, H. (1969) J. Mol. Biol. 42, 1–14.