# CALCIUM- AND ATP-DEPENDENT CHANGES IN MYOSIN MASS DISTRIBUTION OF GLYCERINATED RABBIT PSOAS MUSCLE

R.A. CHAPLAIN* and U. GERGS
Department of Biocybernetics
Advanced College of Technology
301 Magdeburg, Kleiberweg 14, GDR

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Summary. The density distribution associated with two characteristic equatorial reflections of the X-ray diagram indicates a movement of myosin cross-bridge towards the lattice position occupied by the actin. The extent of this mass transfer depends on the concentrations of ATP and $Ca^{++}$ in the medium. As cross-bridges are still moving away from the myosin filament backbone in fibres stretched to a sarcomere length where the two sets of filaments no longer overlap, simply on adding low levels of $Ca^{++}$ ions, this suggests a $Ca^{++}$-sensitive regulatory system on the myosin.

Within the double hexagonal array the thick myosin-containing filaments are situated at the lattice points while the thin actin-containing filaments occupy the trigonal positions of the myosin lattice (1,2). The lateral spacing of the filaments gives rise to two strong equatorial X-ray reflections, the 1,0 and the 1,1, from which it is possible to derive not only the interfilament distance but also the mass of protein associated with the actin and myosin filaments (3,4). Mass transfer is associated with the movement of the myosin cross-bridges from their resting detached position towards the actin filaments when the muscle is stimulated or goes into rigor (4-6). The decrease in mass on the myosin filaments and the corresponding increase in mass on the actin filaments as the cross-bridge heads move into the vicinity of the actin increases the intensity ratio I 1,1/ I 1,0 (3,4). Using glycerinated rabbit psoas muscle an attempt has been made to simulate different functional states likely to occur in actively contracting muscle by recording the intensity ratio of

*Present address: Department of Physiology, University of Mainz, FRG.

the two equatorial reflections at varying concentrations of $Ca^{2+}$ ions and different ATP levels. To test for possible direct effects of $Ca^{2+}$ at the level of the myosin cross-bridges as suggested by the studies of Werber and Oplatka (7) glycerinated fibres have been additionally extended to a sarcomere length where the two sets of filaments no longer overlap.

## Material and Methods

Rabbit psoas muscle was glycerinated *in situ* (8), only that 5% bovine serum albumin was additionally present in the 50% glycerol solution which improves the preservation of the fine structure, possibly by reducing the level of fatty acids set free in the course of glycerination. The muscle was then stored for 4-8 days at -15°C. In the experiments 25 muscle fibres were used, sampling only fibres with a diameter between 50 - 65 µm. The ends of the fibres were glued onto two glass rods, one of which was connected to a strain gauge tension transducer. The fibres were immersed in solutions containing 20 mM Tris-maleate-5 mM K-phosphate, pH 6.8, between 0.05 - 10 mM Mg-ATP, 2 mM phosphoenolpyruvate, 20 µg pyruvate kinase, and 10 mM Na-azide to inhibit the non-myofibrillar ATPase. KCl was added to give an ionic strength of 0.12. The free $Ca^{2+}$ level, stabilized by a CaEGTA/EGTA buffer, was calculated using the association constants quoted by White and Thorson (8). The central portion of the fibres was sandwiched between two Mylar windows to allow the X-ray beam and the laser light to pass through. A mirror-monochromator camera and a Huxley-Holmes-type rotating anode X-ray rube (loading 1.1 kW) was used. The intensities on the 1,0 and 1,1 innermost reflections were recorded for 10 min periods by a proportional counter. For a specimen-counter distance of 75 cm the X-ray tube focus and the quartz crystal were 40 cm apart and the focal distance was 90 cm. The X-ray beam was collimated before the specimen to 150 µm x 180 µm. At the counter the beam had a rectangular shape of about 100 µm x 300 µm. A lead mask allowed the intensities at 1,0 and 1,1 to pass through specific apertures. The 1,0 reflection was separated from the central scatter by placing a guard edge on the counting side close to the specimen.

The counting rate in the absence of muscle was subtracted as the background. This may well represent an overestimate of the contribution made by the background to the measured intensities. Even in the very stretched muscle the camera background was never higher than two-thirds of the total count.

Sarcomere lengths were measured with a Zeiss-Helium-Neon laser. At 2.6 $\mu$m (which corresponds to rest length (3)) the spread was 3% over the whole region, increasing to 5-15% when the sarcomere length was 4.5 $\mu$m.

## Results

In agreement with previous work (3,4) on living muscle the ratio of intensities I 1,1/I 1,0 exhibited a minimun value of $0.55 \pm 0.07$ (mean $\pm$ S.D.) at 10 mM Mg-ATP and $10^{-9}$ M $Ca^{2+}$, defined as relaxing condition. In the absence of ATP this ratio was as high as $2.5 \pm 0.2$ (mean $\pm$ S.D.), which is only slightly higher than the ratio obtained for intact rabbit psoas in rigor (3). To facilitate a comparison of data obtained on different fibre bundles the difference between the two extreme ratios characteristic of the respective fibre bundle was always defined as unity and the intensity ratios at the intermediate ATP levels and the different $Ca^{2+}$ concentrations have been expressed as fractions thereof. To display the data on a comparable scale, the muscle tension, which was zero under relaxing conditions and $110 \pm 17$ mg/fibre (mean $\pm$ S.D.) in rigor state, has equally been normalized against the rigor value.

The data shown in Fig. 1 reveal clearly that all conditions leading to an increase in muscle tension also increase the ratio of I 1,1/I 1,0. Higher intensity ratios of the two equatorials can be obtained as the ATP level is gradually decreased from 10 mM to 0.1 mM, even in the virtual absence of $Ca^{2+}$ ions. Increasing the $Ca^{2+}$ level from $10^{-9}$ to up $10^{-4}$ M induces an additional shift in the intensity ratio towards the rigor pattern. At 5 - 10 mM ATP, which corresponds to the ATP level in living muscle when due account is taken of the intrafibrillar diffusion in glycerinated fibres, even at $10^{-5}$ - $10^{-4}$ M $Ca^{2+}$ which will be maximally activating for the ATPase (9), there is only a relative increase of 20 - 37% in the I 1,1/I 1,0 ratio.

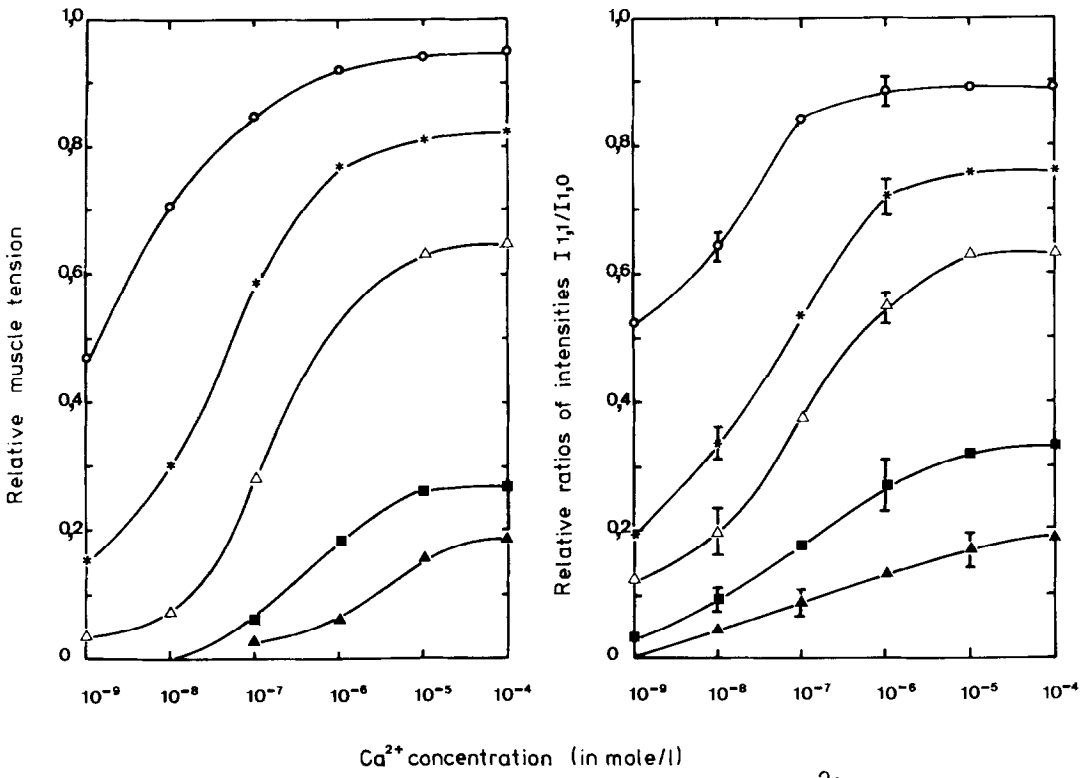


Figure 1. The effect of varying levels of ATP and $Ca^{2+}$ ions on tension development and myosin mass redistribution between myosin and actin filaments. The concentrations of Mg-ATP in the medium were as follows: 0.1 mM ( o ), 0.5 mM ( * ), 1 mM ( Δ ), 5 mM ( ■ ) and 10 mM ( ▲ ). The pattern at the 1,0 and 1,1 equatorial reflections was recorded only at the intensity peaks and not for the regions in between them as the original recordings on film showed essentially no change in the shape of the peak. The maximal counting rate (corrected for the camera background) was 360 000 counts per minute for the first equatorial. Error bars are given for the 1,1/1,0 intensity ratios.

This corresponds to a redistribution of myosin mass equal to one-third of the rigor movement. These changes are somewhat lower than those observed by Huxley and Haselgrove (4) for contracting frog sartorius muscle, which may reflect genuine differences between frog and rabbit muscles or between live and glycerinated fibres. If one compares the relative ratios of I 1,1/1,0 with the tension pattern there is a definite tendency at the lower $Ca^{2+}$ for the intensity ratio to increase to a much larger extent than the muscle tension.

As there exists suggestive evidence in the literature (10, 13) that the initial movement of the myosin cross-bridge may be unaffected by the presence of the actin filaments, the effect of ATP and $Ca^{2+}$ has been investigated at a mean sarcomere length of 4.5 µm, where the two sets of filaments no longer overlap. The absence of any significant amount of actin-myosin interactions is emphasized by the finding that only 0.2 - 0.9 mg tension are developed when such highly stretched fibres were allowed to go into rigor and that this tension did not increase with time.

Consistent with earlier work (2,4) the intensities of the 1,0 and 1,1 reflections have decreased considerable below their values at rest length. Further, I 1,1 has been reduced to a much greater extent than

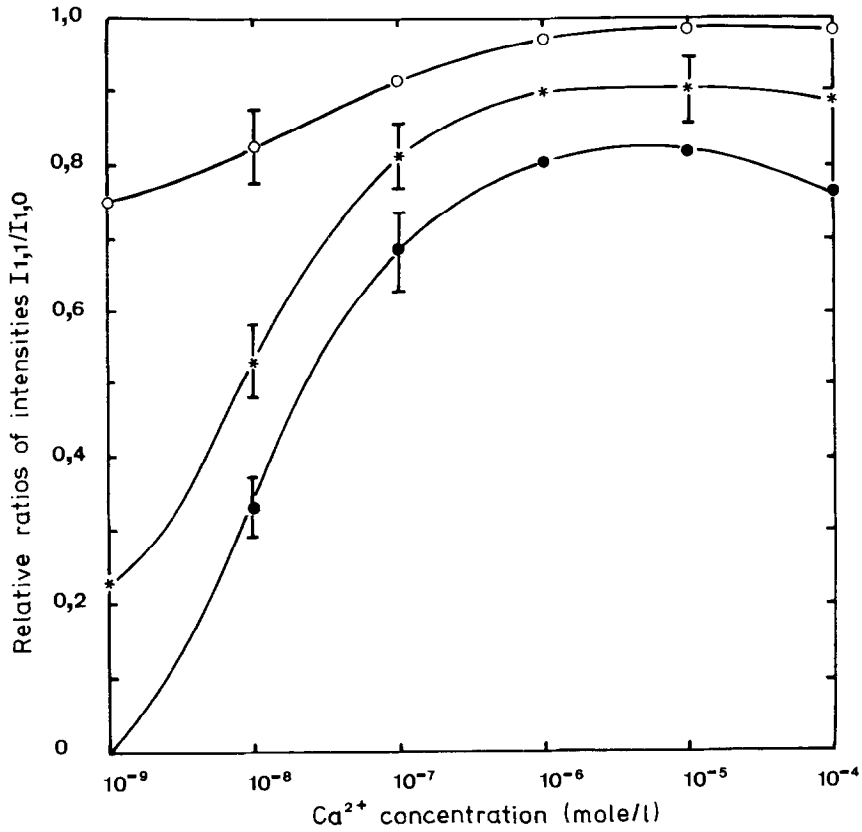


Figure 2. Myosin mass movement in highly stretched rabbit psoas fibres. The Mg-ATP concentrations in the medium were 0.05 mM ( o ), 2 mM ( * )and 10 mM ( ● ), with the free $Ca^{2+}$ level given in the Figure. Error bars are included.

I 1,0. Thus, disorder in the lattice may be a factor contributing to the change in the apparent peak intensity. Under relaxing conditions the ratio of I 1,1/I 1,0 is now only 0,11 $\pm$ 0.03 (mean $\pm$ S.D.), the 1,0 spacing being 336 $\pm$ 5 Å . In the complete absence of ATP the ratio I 1,1/ I 1,0 assumed a value of 4.2. To provide a comparable scale for the sets of data obtained at 2.6 /um and 4.5 /um sarcomere length the ratios of I 1,1/ I 1,0 under relaxing conditions have again been defined as zero and those in rigor as unity and the intermediate values have been normalized.

When increasing $Ca^{2+}$ levels are added to highly stretched fibres the ratio of I 1,1/ 1,0 still increases (Fig. 2), less $Ca^{2+}$ being required at lower ATP levels to give the marked intensity increase of the 1,1 reflection. The results are consistent with a major transfer of myosin mass away from the myosin filaments at fully relaxing Mg-ATP concentrations when the $Ca^{2+}$ level is raised to as little as $10^{-8}$-$10^{-7}$ M. The intensity changes in Fig. 2 suggest that $Ca^{2+}$ in some way overcomes the effect of ATP and that it does so much more effectively than in the case of complete filament overlap (Fig. 1).

## Discussion

The effect of increasing $Ca^{2+}$ levels points to the regulatory function of the troponin-tropomyosin system (11,12) with more actin sites becoming available for interaction with myosin cross-bridges at higher $Ca^{2+}$ (13). It could be argued that the effect of ATP can be explained by the concept of Bremel and Weber (14) in that this prevents the formation of rigor complexes and hence a displacement of the tropomyosin by attached myosin heads. However, as the increase in the I 1,1/I 1,0 ratio persists also in fibres stretched to the non-overlap point, a direct action of both ATP and $Ca^{2+}$ on the myosin head appears more likely. Low levels of $Ca^{2+}$ ions seem to be able to induce a movement of the cross-bridges towards the trigonal positions of the lattice (Fig.2). Together with Huxley's studies (10,13) on highly stretched frog semitendinosus, which demonstrated an effect of stimulation and hence $Ca^{2+}$ on the myosin layer-lines, this may be taken to suggest the existence of a $Ca^{2+}$-sensitive system at the level of the myosin cross-bridge. In

agreement with this postulate, it has been shown recently that one of the light chains from rabbit myosin binds $Ca^{2+}$ ions and that removal of this ligth chain causes a decrease in the $Ca^{2+}$ sensitivity of actomyosin (7). Such a $Ca^{2+}$-sensitive system may be the evolutionary equivalent to the myosin-linked regulatory system described for molluscs and arthropods (15). In this respect it is of interest that a similar analysis of the equatorial changes in glycerinated insect fibrillar muscle has demonstrated that upon addition of $Ca^{2+}$ the cross-bridges move out to the environment of the actin without necessarily attaching to the actin helix, as indicated by the lack of changes in the characteristic X-ray layer lines (16).

In contrast to the troponin system which seems to operate at $Ca^{2+}$ concentrations above $10^{-7}$ M (11,14), the system on the myosin appears to function at lower $Ca^{2+}$ levels (Fig.2). It is of interest, that the functional Ca-binding site on the myosin light chain involved in the interaction with actin has indeed a binding constant of $102 \cdot 10^{6}$ $M^{-1}$ (17). A physiological function of such a $Ca^{2+}$-sensitive cross-bridge movement would be to block all ATPase activity in resting muscle. In view of the high background ATPase observed with isolated proteins and myofibrils at least one in seven actin monomers may not be masked by the tropomyosin (12). Consistent with this hypothesis is the finding that when $Ca^{2+}$-binding to troponin is abolished by the binding of anti-troponin to rabbit psoas fibres the ATPase in presence of $2 \cdot 10^{-7}$ M $Ca^{2+}$ is inhibited by only 15% (18).

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