CHANGES IN THE PITCH OF THE MYOSIN HELIX PRECEDING CROSS-BRDIGE ATTACHMENT IN OSCILLATORY CONTRACTIONS OF FROG SARTORIUS MUSCLE

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Summary. When glycerinated fibres in presence of $5\cdot10^{-7}$ M Ca^{2+} were oscillated at a frequency of 10 Hz, the X-ray intensities at 429 Å and at the small radial position of the 191 Å layer-line change in an inverse pattern. This rearrangement of the pitch of the myosin helix precedes by at least 10 msec the intensity increase on the 59 Å X-ray layer-line, which provides a measure for cross-bridge attachment to the actin. The perfect match of the new pitch of the myosin helix with the pitch of the actin helix may ensure optimal cross-bridge attachment.

To elucidate the kinetics of the cross-bridge cycle during muscular contraction previous investigations have centered on the movement of the cross-bridges on the 143 Å myosin lattice (1,2) and on the attachment of the cross-bridge to the actin filament as indicated by the near-meridional density on the 59 Å reflection (3,4). However, apart from the studies with ATP analogues (5,6), which are consistent with a disorder in the angle of cross-bridge attachment to the actin, little is known on the intermediate conformational states of the myosin which must be associated with the coupling of the ATPase kinetics to the mechanical events. In relaxed frog sartorius the double helical array of cross-bridges originating from the myosin filament gives rise to a well-sampled layer-line of spacing 429 Å which indicates a pitch of 858 Å for the helix of myosin heads on a thick filament (1) with a subunit spacing of 143 Å. In rigor these layer-lines are replaced by diffuse layer-lines at 365 Å and 385 Å, while even during normal isometric contractions the intensities of the 429 Å and 143 Å layer-lines are reduced much below their relaxed values (1), sugges-

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Such change in the pitch of the myosin helix may well have an important role for the efficient control of chemo-mechanical energy conversion. Hence, it seemed a promising approach to investigate the kinetic relationship of the decrease at 429 Å to the increase at the near-meridional part of the 59 Å layer-line which provides a measure for cross-bridge attachment. As the rigor spacing at 386 Å (1) would correspond to a myosin helix with a pitch of 770 Å, normally found in insect muscle (2), it was desirable to uncover any inherent reciprocal relation between a decrease at 429 Å and an increase at about 380 Å. However, the actin helix in frog sartorius equally has a pitch close to 770 Å (7). Although the actin and myosin helices with a pitch close to 770 Å and a first layer-line around 380 Å both give second order layer-lines at about 190 Å, the intensity contributions by the two helical structures can be clearly distinguished. The intensity at the large radial spacing of 0.021 $Å^{-1}$ on the 191 Å axial layer-line reflects the actin-tropomyosin system (7,8), while the intensity at the small radial spacing of 0.011 $Å^{-1}$ would arise from myosin heads oriented on a helix with a pitch of 770 Å (8).

Material and Methods

Bundles of 15 frog sartorius fibres glycerinated *in situ* by the method of Abbott and Chaplain (9) were used after 6-10 days storage in 50% glycerol - 20 mM phosphate buffer, pH 6.8, at -15°C.

The conditions for the mechanical and X-ray experiments have been described previously (10). The fibre bundle (immersed in a solution containing 20 mM Na_2ATP, 10 mM $MgCl_2$, 20 mM K-phosphate buffer, pH 6.8, 10 mM Na-azide and a mixture of CaEGTA/EGTA to give a stabilized Ca^{2+} level of $5 \cdot 10^{-7}$ M or 10^{-9} M under relaxing conditions, made up with KCl to an ionic strength of 0.125) were sandwiched between two Mylar windows to allow the X-ray beam to pass through. The fibres were again glued at a free fibre length of 1 cm onto two glass rods, one connected to a vibrator the other to a tension transducer.

While the total intensity of the off-meriodonal 429 Å reflection was recorded, of the density distributed along the 59 Å layer-line only the density near the meridian up to an equatorial spacing of 0.005 $Å^{-1}$ was sampled (4). The intensity of the 191 Å layer-line was recorded with a position-sensitive counter at the radial spacing of 0.01-0.013 $Å^{-1}$. The total counting period for accumulating counts on each of the three reflections was 20 min. at any interval given.

Using a Zeiss Helium-Neon laser the sarcomere length of the muscle fibres was adjusted to a mean length of 2.2 - 2.3 µm for the oscillatory experiments. In a number of experiments the fibres were stretched under relaxing conditions gradually to a sarcomere length of 4.4 µm where the actin and myosin filaments no longer overlap.

Standard errors (S.E.) of the experiments are given as percentage of the mean (see also error bars in the Figure) with each value being averaged from 12 - 14 experiments.

Results

When glycerinated frog sartorius fibres are oscillated in presence of $5 \cdot 10^{-7}$ M Ca^{2+} at a sinusoidal frequency of 10 Hz (amplitude 100 µm peak-to-peak) the fibres respond to the sinusoidal length changes with delayed tension changes. The oscillatory work performed on the mechanical apparatus was equal to 0.24 ± 0.08 µcal/fibre/min (mean ± S.E.). In parallel to the recording of muscle tension and work the intensity changes on the three X-ray layer-lines were measured.

To relate the observed intensity changes to some internal standard the density variations at 59 Å have been expressed as fractions of the maximal intensity under rigor conditions where most of the cross-bridges will have become stably attached to the actin (3,4,11). The intensities on the 191 Å and 429 Å layer-lines are given as percentage deviations from the intensity values recorded at $5 \cdot 10^{-7}$ M Ca^{2+} under isometric conditions. No intensity fluctuations on any of the three layer-lines could be detected under relaxing conditions.

Tension development and cross-bridge attachment as measured by the 59 Å density vary essentially in phase (Fig. 1). In contrast, the intensity increase on the 191 Å layer-line and the inverse reduction at 429 Å are leading the changes at 59 Å by about 10 msec. Further, when

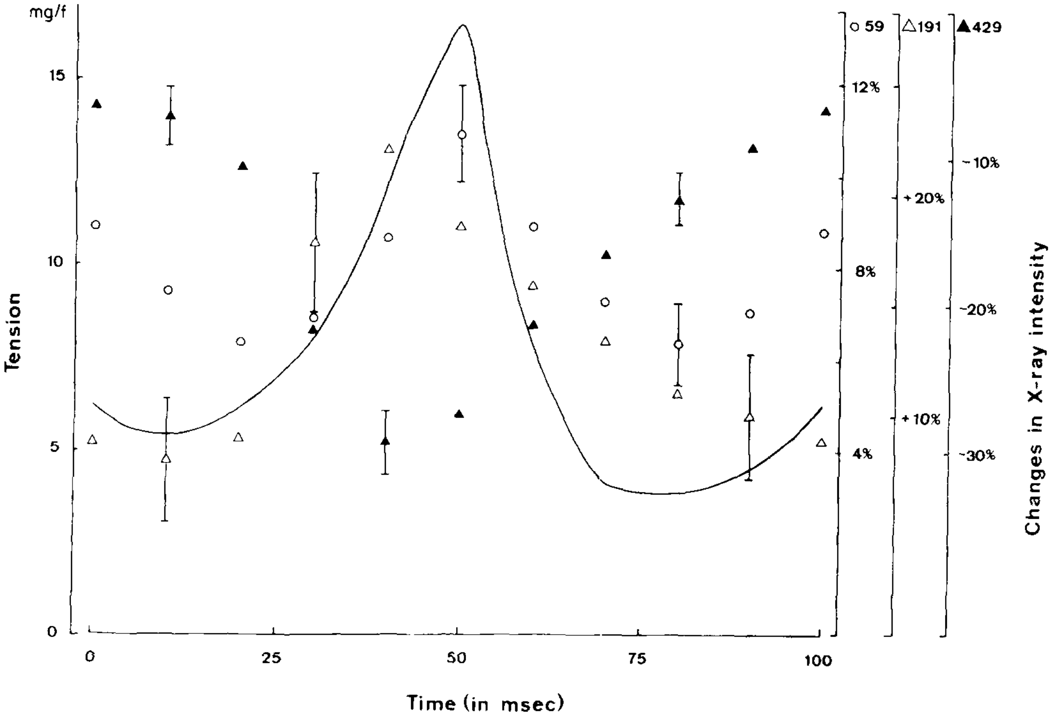

Figure 1. Time course of the changes in the pitch of the myosin helix and in cross-bridge attachment during oscillatory contraction-relaxation cycles of frog sartorius muscle. The tension record (in mg/fibre) is drawn as a continuous line, while the intensity changes are indicated by the respective symbols for 429 Å (▲), 191 Å (△) and 59 Å (o). The error bars on the data provide some information on the characteristic standard error at each of the three layer-lines.

the maximum number of cross-bridges has become attached half-way through the oscillation cycle the 191 Å intensity is already declining again, although the reduction in X-ray intensity at this axial spacing seems to be slower than the fall in muscle tension (Fig. 1).

The absolute magnitude of the changes at 191 Å and 429 Å is well above the static level. Sinusoidal stretching favours the change in the pitch of the myosin helix. Hence, this conformational change may well be related to the phenomenon of stretch activation observed in ATPase and mechanical studies of glycerinated frog sartorius (12,13).

When compared to the rigor conditions the total number of cross-bridges attached to the actin at these low Ca^{2+} levels seems to be rather small and certainly less than the 40-50% reported during isometric contractions under tetanic stimulation (14).

The fact that the intensity change on the 429 Å spacing is divorced from cross-bridge attachment and active tension development can be demonstrated with fibres stretched to the non-overlap point where the cross-bridge heads no longer find an actin binding place. When the sarcomere length was extended to 4.4 µm at 10^{-9}M Ca^{2+} (simulating the relaxing conditions) it could be shown that changes occurred at 429 Å under static conditions simply by adding $5 \cdot 10^{-7}$M Ca^{2+} to the medium. Although the muscle tension increased by as little as 0.5 mg/fibre and the near-meridional density at 59 Å was raised only by 3 ± 0.8% (mean ± S.E.), there was a drop of 43 ± 6.7% (mean ± S.E.) in the 429 Å intensity as compared to the relaxed conditions (compare (8)).

Discussion

As the inverse changes on the 191 Å and 429 Å layer-lines precede those at 59 Å and the 429 Å intensity is reduced under isometric conditions upon addition of Ca^{2+} ions even though the cross-bridge heads in highly stretched fibres cannot find a binding place on the actin, it seems likely that the structural change is induced at the level of the unattached cross-bridge which has already split its bound ATP (15). A possible mechanism would be that the free energy of ATP hydrolysis, transferred through dipole excitation and resonance interactions, is utilized to induce a shortened state of hydrogen or H-bridges in the α-helical backbone of the cross-bridge stalk. Variations in H-bridge length of up to 0.24 Å have been suggested for other helical structures (16). Such a shortening of H-bridge length would be accompained by a "twisting" of the myosin helix in the cross-bridge stalk (maximally by about 2π). As the result of the torsional strain the changes in the helix pitch of the cross-bridge stalk are transmitted to the filament backbone to the effect that the superhelix of the left-handed helical tracks of cross-bridge origins along the myosin filament shaft (1,17) is twisted in a right-hand turn until the pitch of the helix has decreased from 858 Å to 770 Å. This would be consistent with the cooperative reorganization of the helical arrangement of cross-bridges, as indicated by the loss in intensity of both the 429 Å and 143 Å reflections (1,8) and also with the observed reduction in myosin filament length during active muscle contractions (16,17). The effect of this conformational change may be to achieve a perfect match of the helix of cross-bridges on the myosin filament with the pitch of the actin helix which may be prerequisite for optimal cross-bridge attachment. The finding that in the time course of the oscillation cycle the maximal change in the pitch of the myosin helix precedes the peak value of cross-bridge attachment by about 10 msec (Fig. 1), and that this structural change can be induced by Ca^{2+} ions in the complete absence of filament overlap, indicates that the shortened pitch of the myosin helix must be able to exist as a stable conformation for a certain length of time. If the binding von Ca^{2+} to one of the myosin light chains (20) would somehow activate the splitting of ATP this could provide an energy source for the conformational changes still occuring in the absence of any cross-bridge attachment. During the time period where the cross-bridge has split its bound ATP but has not actually attached to the actin, the postulated energy-rich metastable state of the shortened H-bridges in the cross-bridge stalk may be stabilized by such forces as long-range dipole-dipole interactions (21) between amino acid side chains and with water molecules. Once the myosin head becomes attached the H-bridges would re-extend and the stored torsional energy may be used to induce an angling of the cross-bridge head (11) and hence a shearing force on the actin. Under relaxing conditions the stored energy would be gradually dissipated and the pitch of the myosin helix returns to its original length. This would be in agreement with the relatively slow return of the relaxed myosin layer-line pattern following tetanic stimulation (8) and the ability of relaxing muscle to support work performance from an apparent energy storage (22). Attached cross-bridges would tend to stabilize the shorter pitch of the myosin superhelix, hence the slower decline of the 191 Å intensity in Fig. 1, until it may become under rigor conditions the most stable conformation, giving rise to the characteristic 386 Å layer-line (1).

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