ESRF

Experiment title: STRUCTURAL DYNAMICS OF MUSCLE CONTRACTION: A COMBINED MECHANICAL AND TIME RESOLVED X-RAY DIFFRACTION STUDY ON SINGLE MUSCLE FIBRES

Experiment number: LS-347

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Report:

Experiments were done at BL4 with a monochromator/mirror X-ray camera. Single fibres, dissected from the tibialis anterior muscle *of Rana* temporaria just before the experiments, were mounted horizontally in a trough containing Ringer solution at 4°C between a fast force transducer (resonant frequency 40-60 kHz) and a loudspeaker coil motor. Two mica windows carrying the electrodes were moved as close as possible ($\approx 600\,\mu m$ apart) to reduce the X-ray path through the solution. X-ray exposure was limited to the period of data acquisition by a fast shutter (switch time ≈ 5.4 ins). The X-ray pattern was recorded by means of a two-dimensional gas-filled detector and associated data acquisition system, with a specimen-detector distance of 10 m. Data analysis was performed using the BSL/XOTOKO packages provided by SERC Daresbury Laboratory.

The beam was operating in 16 bunch mode. To achieve the maximum efficiency in the 2D gas-filled detector we had to reduce the flux to about 20% of the maximum achievable. To minimise radiation damage on the fibre we had to further reduce the flux by a factor of 2 and move the fibre longitudinally by 0.5 mm after each tetanus. In each fibre good quality patterns were collected for 12-15 tetani with a total exposure time of 6-8 s.

Experimental protocol. The fibre was titanically stimulated using alternating 0,5 ms pulses of 1.5 times the threshold voltage at a frequency of *20 Hz* for 550 ms. At the plateau of the isometric tetanus (300 ms after the start of stimulation) a constant velocity shortening was imposed by the loudspeaker motor either at a velocity 0.8 V_0 (V_0 is the maximum velocity of shortening) or at 0.3 V_0 (the velocity at which the muscle fibre develops the maximum power). Following shortening of 5% of the fibre length, the force redeveloped in isometric conditions. The diffraction pattern of the fibre was recorded either with 50 ms time frames before the start of stimulation and at the tetanus plateau, or with 10 ms time frames during the rising phase of the tetanus and during force redevelopment and 5 ms time frames during steady shortening.

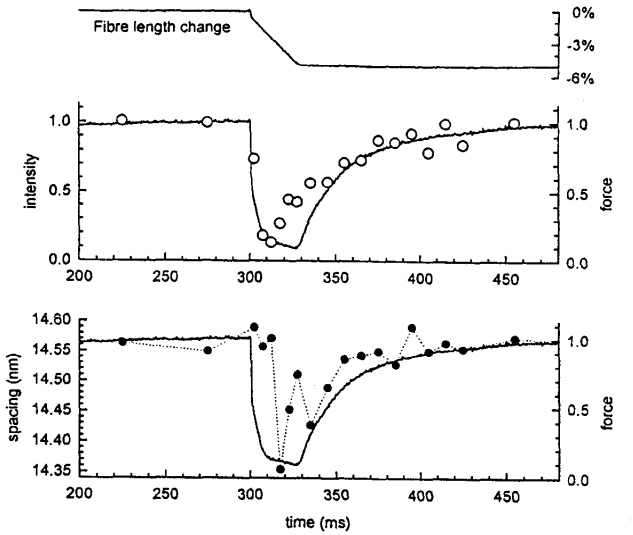

Fig. 1. The intensity (open symbols) and spacing (filled symbols) of the third order meridional reflection are plotted superimposed on force during the response to shortening at the velocity of 1.7 fibre length/s (0.7 V_0). Force and intensity are relative to the isometric plateau value. Zero time is the start of stimulation. Data averaged from two fibres (26 tetani). The first two X-ray data points and the last one are 50 ms time frames. The six data points during shortening are 5 ms time frames; the sampling rate during force redevelopment following the end of shortening is at 10 ins/frame. The intensity is calculated by integrating between 1/80 nn on either side of the meridian and 1/14 and 1/15.2 nm along the meridian after subtracting a linearly fitted background. The spacing was measured using the centre of mass of the peak after background subtraction with a program provided by Alex Stewart.

Results. Changes in intensity and spacing of the third-order myosin meridional reflection (14.3 rim), arising from the axial mass projection of the myosin heads, could be precisely measured by signal averaging the 2D patterns from seven fibres. Force development is accompanied by an increase in axial spacing of the reflection from 14.34 nm (rest value) to 14.57 nm (tetanus plateau value). The reflection intensity decreases within 50 ms, due to the loss of resting crystallographic order, then increases with a time course that almost superimposes force development and spacing change. According to previous results during the first experiments at BL4 (Debbie et al. ***J. Muscle Res. Cell Motility***, in press) steady shortening at 0.3 V_0 decreases the force and the intensity of the reflection to about 60% of their respective isometric values, but does not affect the spacing of the reflection by more than 0.3%. Steady shortening at a velocity 0.7 V_0 (Fig. 1), which decreases the force to - 0.1 the isometric plateau value, produces a sudden reduction of intensity proportional to the drop in force and a later partial recovery. The spacing of the reflection remains 14.57 nm within the first 10 ms of shortening (which corresponds to an amount of sliding of 17 nm per half-sarcomere) and then drops to a value intermediate between the rest and the tetanus plateau value. Both intensity and spacing recover the plateau values during the isometric force redevelopment.

These results are consistent with H.E. Huxley's hypothesis ***(Science*** 164, 1356, 1969) of head tilting during the execution of the working stroke (Irving et al. ***Nature 357,*** 156, ***1992;*** Piazzesi et al. ***Biophys. J 68, 92s, 1995)*** and support the view that the increase in spacing from 14.34 to 14.57 is due to myosin heads attaching to actin in a strongly bound configuration. During shortening at a moderate velocity (which elicits the maximum power output) myosin heads undergo strong binding to actin as in the isometric contraction; during shortening at high velocity, once the working stroke of those heads attached in the original isometric conditions is completed (beyond 15 nm per half-sarcomere of shortening), freshly interacting heads have less opportunity for binding strongly to actin.