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-870
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Report:

Experiments during LS-870 (March 98) were aimed at determining whether the changes in the quasi-helical arrangement of the myosin heads in the thick filament and the 1.5 % increase in their axial spacing during the transition from rest to isometric contraction of the muscle fibre (LS-529; Reconditi et al. 1998; Piazzesi et al. 1998) are related to activation per se or to binding to actin and force generation. We recorded static patterns from single fibres at different sarcomere length between 2.2 μm (full overlap between myosin and actin filaments) and 3.6 μm (no overlap) at rest and at the isometric tetanus plateau. In this way it is possible to isolate the effects of activation which are still present at the longer sarcomere lengths where actin binding and force are abolished in the region of non overlapping filaments. An unprecedented resolution from both resting and contracting fibres was attained as follows: *i*, fibres were mounted vertically in a specially devised trough, so that the meridional axis was parallel to the smallest dimension of the x-ray beam; ii, two-dimensional patterns were collected on a storage phosphor image plate (IP, A3 size), so as to minimise the point spread function of the detector.

Experimental protocol Single fibres from the tibialis anterior muscle of *Rana temporaria* were mounted horizontally in a trough containing Ringer solution at 4 °C between a strain gauge force transducer and a loudspeaker coil motor. Two mica windows carrying the electrodes were moved as close as possible (~600 μm apart) to reduce the X-ray path through the solution. The system was carried on a plate mounted on the movable stage of a Zeiss ACM microscope for measurements of the fibre and sarcomere dimensions. The plate was then mounted vertically on the beam line with the transducer at the top and the motor at the bottom. The physiological solution was maintained in the trough by means of a perspex cover sealed with silicone grease. X-ray exposure was limited to the period of data acquisition by a fast shutter (switch time ~5.4 ms). Specimen-detector distance was 9 m so as to collect meridional reflections up to the sixth order. The beam was operating in 2/3 filling mode at 200 mA current. In each fibre good quality patterns were

recorded with a total exposure time of 12 s. At each sarcomere length 2D patterns were collected at rest and at the plateau of a 2 s isometric tetanus adding three 2 s frames in each of the two conditions. To reduce the effects of the long time necessary for extraction-insertion of IP, two images were collected on each IP by masking half of the IP with a blanking plate inside the beam pipe. IP were scanned with 100 pm spatial resolution. Data analysis was performed using the BSUOTOKO packages provided by SERC Daresbury Laboratory.

Results The diffraction patterns showed splitting of meridional reflections either at rest or at the plateau of isometric tetanus, due to the interference between myosin heads in the two halves of the muscle sarcomere (LS-719). At any sarcomere length, only the meridional reflections at 14.56 nm (M3) and 7.3 nm (M6) remained strong upon activation. At rest the integrated intensity of M3 and M6 did not change with sarcomere length, while during isometric contraction it reduced linearly with reduction of overlap (Fig. 1, left panel). This indicates that these signals originate solely from heads attached to actin in the region of overlapping filaments, very likely because of the high mobility of detached heads. The splitting of these reflections in the active fibre, consistent with an interference distance at full overlap of about 860 nm (which exactly matches the separation of the centres of the region of myosin filament containing myosin heads, LS-719), does not show significant changes with reduction of overlap (Fig. 1, right panel), as if the increase in the interference distance with sarcomere length, due to the increase in separation of the two arrays of attached heads in the two halves of the sarcomere proceeds in steps equal to integer multiples of the spacing of the diffractors. This indicates that in the region of no overlap the axial spacing between myosin heads is the same as in the region of overlap, and thus that the change of the arrangement of myosin heads on the thick filament accompanying the development of isometric force is due to structural changes in the backbone of the filament irrespective of head attachment to actin.

These results represent a breakthrough for a fundamental question in muscle biology which could not have been solved with whole muscle studies for many years (Haselgrove, 1975). The definitive answer has been found as a result of the combination of our single fibre mechanics, which allows precise sarcomere length measurements, with the unique quality of optics at ID2 which made possible to use the interference effect to attain an unprecedented spatial resolution.

Fig. 1. *Left:* relation between integrated intensity of M3 reflection during isometric tetanus (I(M3)a) and sarcomere length. For each fibre and sarcomere length, $I(M3)_a$ is normalised for the resting M3 intensity (I(M3),) in the same fibre. *Right:* spacing of the two main subpeaks of M3 reflection in the active fibre as a function of sarcomere length.

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