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-719
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Report:

Experiments during LS719 (November 1997) were designed to maximise spatial resolution of the myosin-based meridional reflections from single muscle fibres at rest and at the plateau of the isometric tetanus. The aim was to determine changes in the structure of thick filament and myosin heads accompanying activation and force generation. 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), so as to minimise the point spread function of the detector (Fig. 1).

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 (x600 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 7 m so as to collect meridional reflections up to the sixth order. Data analysis was performed using the BSL/OTOKO packages provided by SERC Daresbury Laboratory. 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.

Results 2D patterns were collected at rest and at the plateau of a 2.2 s isometric tetanus, adding three 2 s frames in each of the two conditions. Image plates were scanned with 100 μm spatial resolution. The diffraction patterns collected from four fibres either at rest or at the plateau of the isometric tetanus

showed splitting of several meridional reflections. The separation between sub-peaks was in the range 700-1000 nm, as expected for interference between myosin heads in the two halves of the muscle sarcomere.

In the resting fibre the interference distance was 705 nm, which is shorter that the separation of the centres of the two regions of the myosin filament that contain myosin heads. However it almost coincides with the separation of the two regions of the myosin filament in which C-protein is also present (Malinchik and Lednev, 1992), suggesting that the myosin based-reflections indexing on a periodicity of 42.9 nm arise from this region of the filament, in which there is a quasi-helical arrangement of the myosin heads.

At the plateau of the isometric tetanus the myosin-based layer lines and the meridional reflections at 2 1.4 mn, 10.69 nm and 8.58 nm disappeared. The meridional reflections at 14.56 nm and 7.3 nm remained strong, but their periodicity increased by 1.5% compared with that at rest. The quasi-helical arrangement of myosin heads characteristic of the resting state was completely absent, and was replaced by a strong axial periodicity based on a spacing of 14.56 nm. The clear splitting of this reflection in the active fibre (Fig l., right panel) corresponds to an interference distance of 860 nm, exactly matching the separation of the centres of the entire regions of the myosin filament that contain myosin heads. Thus, in contrast with the resting structure, the whole filament contributes to the myosin-based meridional reflections during contraction.

Comparison of the fine structure of the 14.56 nm and 7.3 nm reflections from contracting fibres also gave new information about the conformation of the myosin heads. The myosin filaments are bipolar so, if the heads are tilted with their actin binding sites towards the centre of the myosin filament, this end of the head has a smaller interference distance. The fine structure of the peak splitting is extremely sensitive to this effect, so that head tilting of the order of 1 nm can easily be detected. The observed fine structure of the 14.56 nm and 7.3 nm reflections was consistent with 3 nm reciprocal tilting between different parts of the head during isometric contraction. Tilting of myosin heads is exactly the motion that is thought to be responsible for force generation in muscle, and the new interference/diffraction approach gives a uniquely powerful tool to investigate it. This approach is only feasible using vertically mounted single muscle fibres on the ID2 beamline.

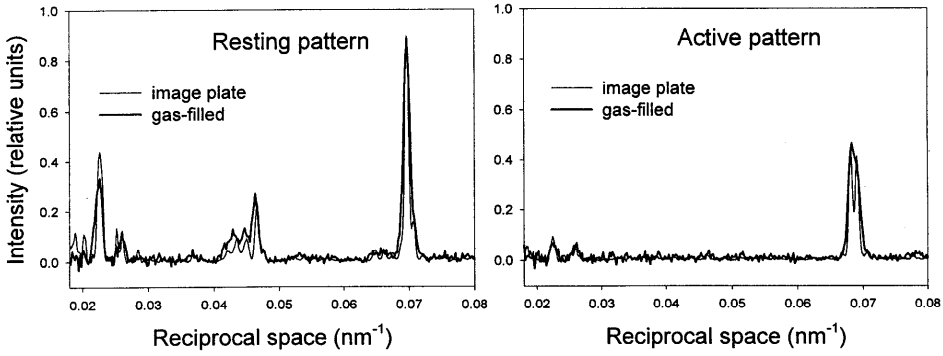

Fig. 1. Meridional diffraction diagrams from a single muscle fibre at rest (left panel) and at the plateau of isometric tetanus (right panel) recorded on both image plate (thin lines) and gas-filled detector (thick lines). The diagrams were obtained by integrating 2D patterns 0.01 nm^{-1} on either side of the meridian in the region from first to third myosin layer line. Total exposure time: gas-filled detector, 10.65 s from 50 ms frames (213 tetani of 600 ms duration from 6 fibres); image plate, 24 s from 2 s frames (3 tetani of 2.2 s duration per fibre, 4 fibres). Note in the active pattern the drop the 2 1.4 nm (forbidden) reflection and the splitting of the reflection at 14.56 nm evident only in the diagram collected with image plate. Average sarcomere length: 2.2 pm, temperature: 6 °C.