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-1403
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Report: The experiments reported here (LS-1403, April 00) are related to the final question at point B of the proposal for a Long Term Project (LS-1403, March 1999), i.e to the possibility to define the extent of the unitary length step in the myosin working stroke by measuring the changes in the interference-fine structure of the meridional myosin based reflections during the synchronous execution of the working stroke elicited by steps in sarcomere length.

The prominent M3 reflection in the small angle X-ray diffraction pattern from a muscle fibre comes from the regular ~14.5 nm repeat of the motor domain of myosin- the myosin head- along the myosin filaments. With the vertical collimation of the X-ray beam at ID2 it has been possible to show that during the contraction of a single muscle fibre the M3 reflection is composed of two closely-spaced peaks (at 14.67 nm, LA, and 14.46 nm, HA), due to X-ray interference between the two arrays of myosins in each thick filament (Linari et al., *Proc. Nat. Acad. Sci.* **97**, 7226-7231, 2000). The interference distance changes when the myosin head binds to actin and drives filament sliding by tilting towards the centre of the myosin filament The method has been used first to measure the change in conformation of the myosin head between the isometric contraction and the rigor state (conformation of the myosin head at the end of the working stroke) observed in the absence of ATP (LS-1013 and LS1262; *ESRF Highlights*, 1999, p.8; paper submitted) and then to characterise the motions of the myosin heads during the quick recovery of force following a step in sarcomere length which synchronises the 1000/s working stroke (LS-1403, October 99). The ratio of the intensity of the high angle peak over that of the low angle peak (I_{HA}/I_{LA}) increases (stretches) and reduces (releases) as expected from the movement of the catalytic domain of the head attached to actin either away from the M-line (stretch) or towards the M-line (release). However, the slope of the relation of I_{HA}/I_{LA} against step size is much smaller than that expected if all the heads attached before the step moved by the amount imposed by the filament sliding. To clear this point it was necessary to record the interference fine structure of M3 reflection at the end of the elastic response in the attached myosin heads, which we achieved in the experiments of April 2000 by collecting 100 μs frames just at the end of the length step.

Experimental protocol: Single fibres from the tibialis anterior muscle of *Rana temporaria* were vertically mounted in a trough containing Ringer solution at 4 °C and at ~2.2 μm sarcomere length between a force

transducer and a loudspeaker coil motor as already described (Linari et al., 2000). To have the required spatial resolution, patterns were collected on a storage phosphor image plate detector (IP, A3 size) placed at 9.8 m from the specimen. The adequate time resolution was attained by using two fast shutters (LS 500, ~ 50 µs switching time) in series in front of the preparation. For collecting the interference change due to the elastic response to a 110 µs length step, the exposure time was precisely synchronized with the end of the step (between 60 and 160µs following the step start) and was recorded by means of a pin diode stuck on the beam stop. For each step size, trains of 40 steps were imposed at the plateau of isometric tetani (Lombardi et al., *Nature* **374**, 553-555, 1995). Data from twenty tetani were added up to a total exposure time of 80 ms per step with unattenuated beam. To distribute the radiation damage, the stage with the fibre was vertically shifted by 200 µm after each exposure. IPs were scanned with 100 µm spatial resolution (Molecular Dynamics). Data analysis was performed using Fit2D (by Dr A.P. Hammersley, ESRF) and Peakfit software package (SPSS Inc.).

Results: Changes in relative intensity and position of the two peaks of M3 reflection for step perturbations in length ranging from +4 to –8 nm show that, as for the active response, the movement of the heads in the elastic response estimated by the interference changes is lower than that expected in relation to the imposed filament sliding. Moreover, following step releases, there is almost no further change in interference-fine structure from the end of the elastic response to the completion of the working stroke. These results can be in part explained by the effect of filament compliance. In fact, the tilting of the lever arm during quick force recovery induces not only the shift of the catalytic domain toward the M line as the actin filament is extended, but also the shift of the neck region toward the Z line as the myosin filament is extended. Both the elastic and the active interference response could be simulated with a structural model of the sarcomere (Linari et al., 2000) with the current estimates of filament compliance, assuming that the observed interference changes are due to two populations of myosin heads: an attached fraction that can undergo a ~10 nm working stroke and a detached or weakly bound fraction. A paper is in preparation.

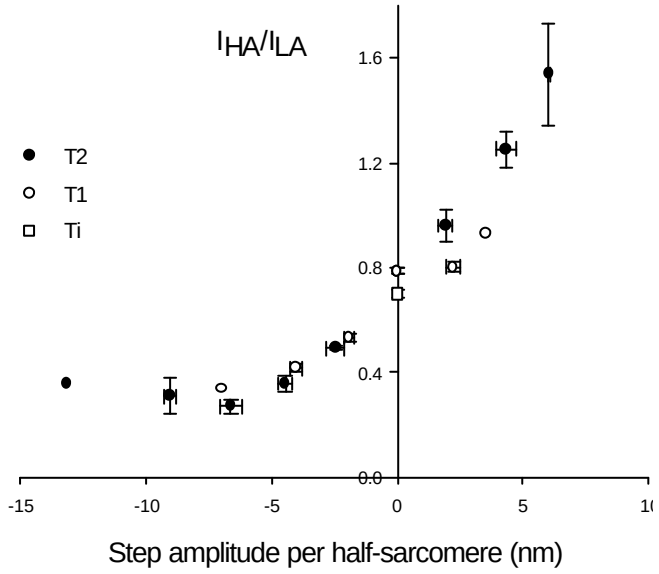

Fig. 1 Ratio between the HA and LA intensities (I_{HA}/I_{LA}) vs. the length step imposed on the fibre. Open circles: at the end of the elastic response (T_1). Filled circles: at the completion of the quick force recovery (T_2). The circle on the ordinate is the control value at the plateau of the isometric tetanus, the square is the value just before the step (T_i). Mean and SE from 25 fibres.

Relative performances of IP and CCD detectors: The IP detector was used for the best spatial resolution but data collection is very inefficient at ID2 because it is necessary to place the IP *in vacuo* and extraction/insertion of IPs and subsequent scanning take several tens of minutes. This problem will be partly circumvent with the planned development of a more flexible camera. We tested the efficiency of collecting the same experimental data on the CCD detector in use at the beamline, which has the image intensifier stage lens coupled to the CCD with a gain that recently has been increased by a factor of 10. However the CCD has a larger PSF than the IP-scanner acquisition system and this makes it unsuitable for interference studies even at 10 m from the specimen.