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, October 99) are related to the final question at point B of the proposal for a Long Term Project (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 during the synchronous execution of the working stroke.

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 unprecedented collimation and fine focus 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 sub-peaks (at 14.67 and 14.47 nm), due to X-ray interference between the two arrays of myosins in each filament(Linari et al., *Proc. Nat. Acad. Sci. USA*, in press). The sub-peak composition of M3 reflection is not affected by increase in sarcomere lengths between 2.2 μm (full overlap between actin and myosin filaments) and 3.6 μm (no overlap), while the M3 intensity decreases in proportion with isometric force and filament overlap, demonstrating that the M3 reflection in the active muscle originates solely from the population of attached heads and that the modification of thick filament structure responsible for the 1.5% change in spacing of the myosin-based meridional reflections from resting to active muscle are independent of cross-bridge formation.

When the myosin head binds to actin and drives filament sliding by tilting towards the centre of the myosin filament (Dobbie et al. *Nature* **396**, 383-387, 1998), the interference distance does change. The method has been used to measure first 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; Reconditi et al., *ESRF Highlights*, 2000; paper in preparation) and then, during the last visit (LS-1403, October 99), to characterise the sub-millisecond motions of the myosin heads responsible for the quick recovery of force (T_2, Fig. 1A, continuous line, see also Huxley & Simmons, *Nature* **233**, 533-538, 1971) elicited by step changes in sarcomere length.

Experimental protocol: Single fibres from the tibialis anterior muscle of *Rana temporaria* were 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. 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 trough was mounted vertically on the beam line with the transducer at the top and the motor at the bottom, to have the meridional axis parallel to the smaller dimension of the beam. To maximise the spatial resolution patterns were collected on a storage phosphor image plate detector (IP, A3 size) placed at 10 m from the specimen. For each step size, trains of 40 steps were imposed at the plateau of isometric tetanic contractions. Each step was followed after 4 ms by a similar step in the opposite direction and the cycle was repeated every 50 ms. A fast shutter (LS500, 100 μs switching time) before the fibre opened from 1 to 3 ms following the shortening or lengthening step. To distribute the radiation damage, the fibre and the stage were vertically shifted by 200μm after each tetanus by using the remote controlled motor at the beamline. Data from twenty tetani were added up to a total exposure time of 1.6 s per step. IPs were scanned with 100 μm spatial resolution (Molecular Dynamics). Data analysis was performed using the program HV written by Dr A. Stewart and Peakfit software package (Jandel Scientific).

Results: Total intensity of M3 reflection (I_{M3}) and mechanical data for step perturbations in length ranging from +6 to –13 nm are shown in Fig. 1A. With respect to the value during isometric contraction, at the end of quick force recovery following the length step, I_{M3} is reduced both for stretches and large releases, as expected if the major axis of the myosin head in the isometric contraction is tilted away from the rigor orientation beyond the point which maximizes the intensity (Irving & Piazzesi, *NIPS* **12**, 249-254,1997).

The interference fine structure at the end of quick recovery changes as expected by the movement of the catalytic domain of the head attached to actin either towards the M-line (release) or away from the M-line (stretch). The ratio of the intensity of the high angle sub-peak over that of the low angle sub-peak (I_{HA}/I_{LA}) increases for stretches and reduces for releases (Fig. 1B), but 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. Since the M3 reflection in the contracting muscle is generated only by myosin heads attached to actin (Piazzesi et al., *J.Physiol.*, **514**, 305-312, 1999; Linari et al, *PNAS*, in press), the results could be explained if either some of the heads undergo rapid (1000/s) detachment /attachment or not all the heads that contribute to M3 reflection respond to the step. To clear what mechanism is implied in the synchronous execution of the working stroke, the interference effect must be recorded at the 100 μs resolution necessary to isolate the purely elastic response simultaneous with the length step.

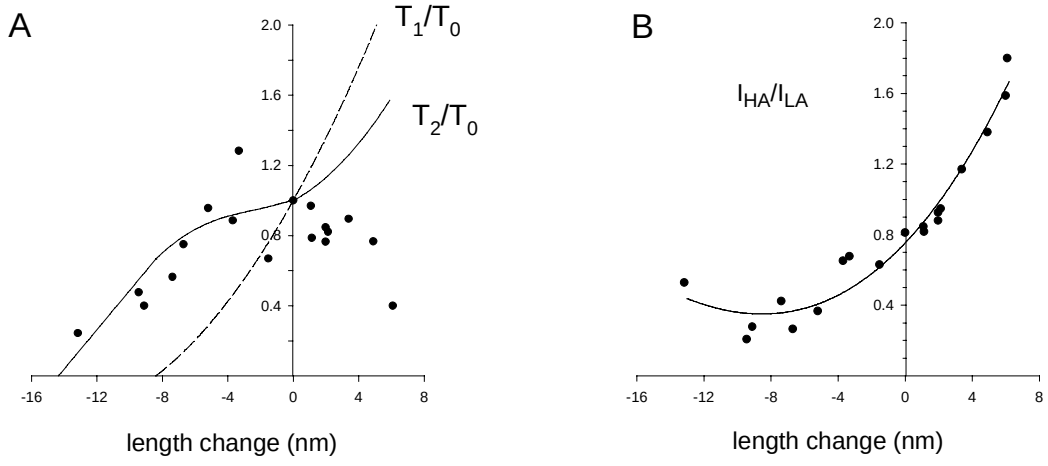

Fig. 1. A: Relation of force attained at the end of the step (T_1, dashed line),force recovered within ~2ms (T_2, continuous line) and I_{M3} at T_2 (filled circles) versus the size of the length step. All data are relative to the value at the isometric tetanus plateau (T_0). B: Relation of I_{HA}/I_{LA} versus step size. The continuous line is the fourth order polinomial fit to data.