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-379 LS-529
Beamline: BL4 ID2	**Date of experiment:** from: 22.05.96 18.09.96 to: 27.05.96 22.09.96	**Date of report:** 26.02.97
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Names and affiliations of applicants (* indicates experimentalists):

*Vincenzo Lombardi
*Gabriella Piazzesi
*Massimo Reconditi
Dipartimento di Scienze Fisiologiche
viale G.B. Morgagni, 63
50134 Firenze - Italy

*Malcolm Irving
*Ian Dobbie
King's College London
26-29 Drury Lane
London WC2B 5RL - United Kingdom

Report:

Methods. Experiments were done at ID2 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 (≈600 μm 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 ms). The X-ray pattern was recorded from the region of the fibre under sarcomere length control by a striation follower, by means of a two-dimensional gas-filled detector and associated data acquisition system. Specimen-detector distance was 6 and 10 m. Data analysis was performed using the BSL/OTOKO 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. In each fibre good quality patterns were collected for 12- 15 tetani with a total exposure time of 6-8 s.

LS379 (May 96)

Experimental protocol and results. We attempted to measure the instantaneous elasticity of the actin and myosin filaments and of cross-bridges in single muscle fibres. The protocol consisted in applying 3kHz length oscillations of 5 nm per half-sarcomere to a fibre during isometric contraction and collecting X-ray data at 20μs time framing with the 2D gas-filled detector. To achieve a good signal-to-noise ratio, these measurements required the summation of at least 12,000 cycles per contraction for a total of 240 ms per frame. Previous experiments made at Daresbury with a much lower spatial resolution and intensity proved the feasibility of the measurements. ID2 at ESRF is greatly superior to the Daresbury beam lines for high resolution spacing measurements because of the much smaller and more parallel beam. The experiments at ID2 failed apparently because the detector data acquisition system could not maintain synchronisation of the data from a very large number of repeats of the sequence of 20μs time-frames, although this is within its specified performance limits. On the other hand data collected in previous experiments at ID2 (LS347) with

5ms time-frames showed the correct temporal discrimination. A detailed report of the results from LS379, including tests of the &ta acquisition system, was sent to the beam line scientists and the problem is being investigated.

LS529 (September 96)

Experimental protocol. At the plateau of the isometric tetanus (300 ms after the start of stimulation) a constant velocity shortening close to the maximum velocity of shortening was imposed by the loudspeaker motor. Following shortening of 6% 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 5 ms time frames during the rising phase of the tetanus and during steady shortening and force redevelopment.

Results. In accordance with previous preliminary experiments at ***ID2*** (LS347, Bösecke ***et al. Pflügers Arch.,*** 1997, in press) we have found that isometric force development is accompanied by an increase in intensity of the third-order myosin meridional reflection, M3, sensitive to movements of the myosin heads, while its spacing changes from 14.34 nm (rest value) to 14.57 nm (tetanus plateau value), and that high speed shortening produces a drop of both the M3 intensity (with the same time course as the tension decrease) and spacing (delayed -10 ms with respect to the tension decrease). The highly collimated beam at ID2 made it possible in LS529 to resolve the M3 reflection into three components (at 14.27 nm, 14.45 nm and 14.68 nm, Reconditi ***et al., Proc. Biophys. Soc. Meeting,*** New Orleans, USA, march 1997, see Fig.1) and measure the respective changes with 5 ms time resolution. The intensity of the 14.27 nm component drops during isometric tension development to 27% of its resting value with a time course that leads tension rise by 20 ms, and does not change during high speed shortening. During the tension development the intensity of the 14.45 mu component follows the same time course as the composite M3 reflection while the 14.68 nm component, which is not present at rest, rises monotonically with tension to its maximum value. During shortening both these components drop with force to about 25% and 10% of their plateau values respectively. Taking into account the interference between myosin heads in the two halves of the myosin filament, the dam can be explained by the presence of two populations of myosin heads: one corresponding to the resting state, with spacing 14.34 nm, and the other to a strong bound force generating state, with spacing 14.57 nm. The possibility to resolve the fine structure of the M3 reflection with such a spatial resolution and frequency response is unprecedented and makes time-resolved SAXS on single muscle fibres at ID2 a unique tool for investigating the structural dynamics of the molecular motor in muscle.

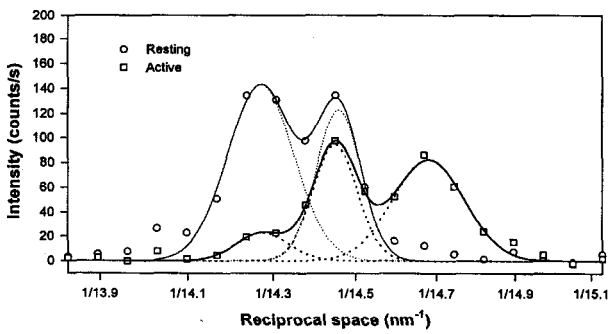

Fig. 1. Meridional diffraction diagrams from a single muscle fibre at rest (circles) and at the plateau of isometric tetanus (squares) recorded in the region of the 3rd order myosin meridional reflection (M3). The diagrams were obtained by integrating 2D patterns 0.01 nm-' on either side of the meridian after subtracting a linearly fitted background. Both sets of data were fitted with three gaussians (thin continuous line: resting fibre; thick continuous line: isometric tetanus). The individual gaussians are shown by the dotted lines.