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-529
Beamline: BL4 ID2	**Date of experiment:** from: 18.09.96 to: 22.09.96	**Date of report:** 26.08.97
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Report:

This report integrates that presented with application LS-719 with the results of further analysis on data collected during LS-529.

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 -1 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 l/3 filling 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 -40 tetani with a total exposure time of -30 s.

Experimental protocol. Tetanic tension was induced at 4 °C by a tram of electrical stimuli at the proper frequency. At the plateau of the isometric tetanus (300 ms after the start of stimulation) shortening at high velocity 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. ln accordance with previous preliminary experiments at ID2 (LS347, Bosecke et al. *Pflügers Arch.* 434:R19,1997) 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 intensity and collimation of the beam at ID2 and the use of the 10 m camera length made it possible to follow the changes in intensity of low order myosin-based reflections (1st order layer line, Ml, and 2nd order meridional reflection, M2) and to analyse the M3 reflection in terms of three components (at 14.25 nm, 14.46 nm and 14.68 nm, Reconditi *et al., XXXIII IUPS Congress,* St. Petersburg, Russia, 30 June-5 July 1997) generated by the interference between the two halves of the myosin filament. 5 ms time resolution was achieved throughout the tetanic tension rise and the high speed shortening. The intensity of the 14.25 nm component drops monotonically during isometric tension development with a half-time of 14 ms, which is similar to the half-time of the drop in the intensity of Ml and M2 reflections and 15-20 ms shorter than that of tension rise. The 14.25 nm component, as the Ml and M2 reflections, is not sensitive to mechanical perturbations such as high speed shortening. During the tension development the intensity of the 14.46 mn component follows the complex time course of the unresolved 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. The complex changes undergone by the intensity and spacing of the M3 reflection during the development of contraction and changes in mechanical conditions are fully explained by the changes in intensity of the three components (Fig. 1). The data can be explained if we assume that the interference function samples two reflections: one due to the disposition of the heads in the resting state, with spacing 14.34 nm, and the other due to the fraction of the heads in the 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.

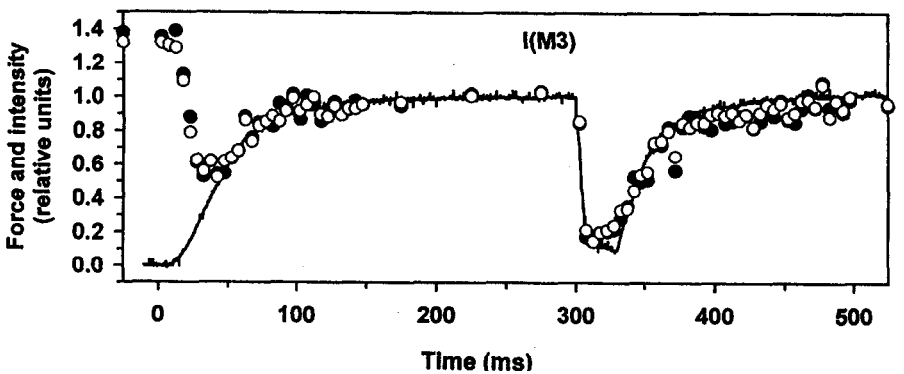

Fig. 1. Comparison between the values of the intensity of M3 reflection obtained either by the integration of the intensity distribution of the unresolved reflection (filled symbols) or by summing the areas of the three gaussians fitted to M3 intensity distribution (open symbols). The continuous line is the force response. Zero time is the start of stimulation. At 300 ms shortening at high velocity was imposed on the muscle fibre, which produced a drop in force from the isometric plateau value (T_0) to -0.15 T_0.