During the experimental session SC-2639 a new set up for time-resolved x-ray diffraction experiments with permeabilised muscle fibres was used. The new apparatus' performance reached all design specifications. The set-up allows us to collect x-ray diffraction pattern from a single, permeabilised muscle fibre or a bundle of 2-3 fibres mounted vertically. The upper end is attached to a force transducer and the lower end attached to a fast motor. The vertical orientation of the sample provides higher meridional spatial resolution of the patterns than for horizontal fibre mounting. The set up is equipped with a remote controlled system to exchange solutions by moving bathing troughs to activate or relax our muscle specimen in a fraction of a second. The challenge is to contain solution in bathing troughs which are devoid of a top or bottom faces, so that fibres are mechanically connected to the motor. Dimensions were set for the solution to remain contained in the trough by surface tension. The fast activation and relaxation protects mechanical and structural integrity of muscle samples so that additional stabilization with EDC (1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide) used in our previous ESRF experiments [1, 2] is no longer necessary. The set up is also equipped with a new Joule temperature jump (T-jump) apparatus that is able to increase temperature of the sample by up to 35°C in a fraction of a millisecond [3]. The fibres were activated with a solution with saturating Ca^{2+} concentration at 0-1°C, suspended in a cold, wet atmosphere at 4°C and then subjected to a T-jump up to a near physiological temperature of 31-34°C. Thus we were able for the first time to study structural properties of permeabilized mammalian muscle fibre which contract at near physiological conditions, in the absence of cross-linking.

Experiments were performed at a fixed wavelength approximately 0.1 nm with the Frelon CCD detector operating at 2048 (vertical) x 256 (horizontal) pixels and a sample to detector distance of 3.5 m, the beam size at the sample was 150 (vertical) x 450 (horizontal) μm (FWHM) with an X-ray flux of *ca*. 3.8×10^{13} photons/s. As the x-ray flux was almost twice that of our previous experiments and the fibres had not been treated with EDC cross-linking, the force developed by the fibres at 31-34°C reached 300 kPa, which resulted in fibre breakage soon after the beginning of an experiment. The problem was overcome by increasing the concentration of Dithiothreitol (DTT) from 10 mM to 80 mM that effectively protects proteins from radiation damage. As a result specimen survived up to 13 activations and x-ray exposure without significant loss of tension.

We had planned to resolve the fine interference structure of the M3 and M6 myosin meridional reflections on both sides of the pattern using a 3.5 m-long camera. Although a clear shoulder was clearly seen on the M3 reflection, the amplitudes and position of its sub-peaks could not be resolved with sufficiently good precision. As a change of camera length and its refocusing could waste beam time we focussed on experiments which require a long camera and studied the movement of myosin heads during isometric contraction upon increases in temperature and during stretch. Precise measurement of the changes in the M3 fine structure during shortening of mammalian muscle fibres contracting at near physiological temperature will need a longer camera. We plan to apply for beam time for performing such experiments.

Two sets of experiments were performed during the SC-2639 session.

1. Duty ratio of myosin motor in muscle at near physiological temperature

In the first set of experiments we compared 2D diffraction patterns during isometric contraction at ~4°C and at 31-34°C with those collected from other segments of the same fibre bundles in rigor and relaxed states (Figs. 1, 2). The aim of the experiments was to obtain a robust estimate of the fraction of myosin heads which are bound to actin strongly and stereo-specifically and produce muscle force at a near physiological temperature.

The intensity of the 1st actin layer line, A1, during high temperature isometric contraction was 29% of its intensity in rigor (Fig. 2). This corresponds to a fraction of myosin heads strongly and stereo-specifically bound to actin of 33-38% [4]. This estimate is close to that obtained in our previous experiments [4] suggesting that structural properties of muscle fibres were not affected by

mild EDC cross-linking used in those experiments.

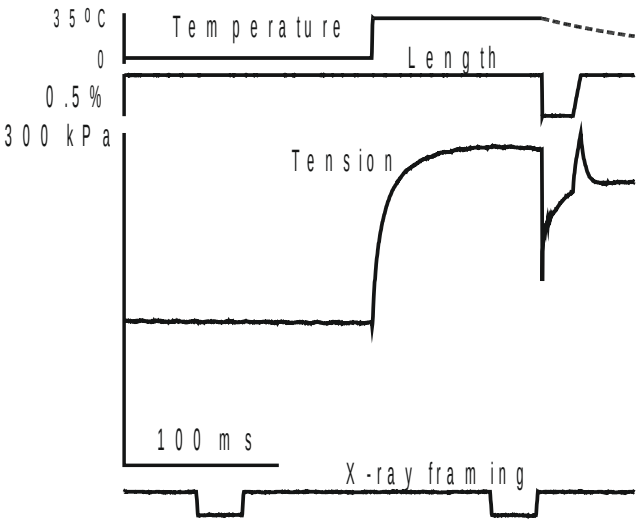

Fig. 1. An example of experimental records: from top to bottom: temperature, length change (in % of total length), tension and x-ray shutter opening.

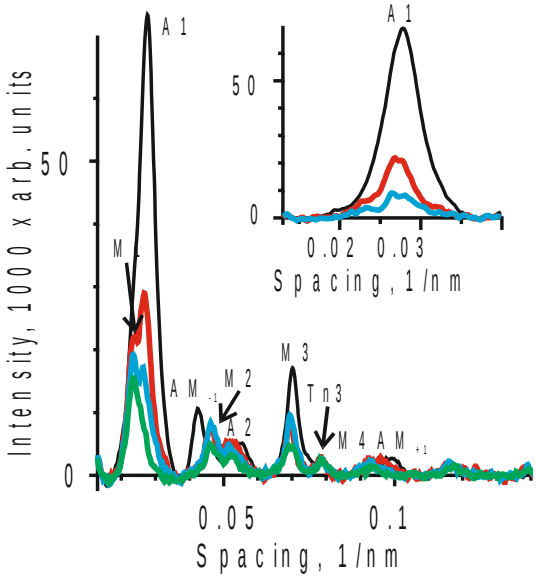

Fig. 2. Meridional profiles of the off-meridional x-ray intensities (integrated in the radial range of 0.018-0.061 nm^{-1}) collected from 3 bundles in rigor (black) and relaxed (green) states and during low (blue) and high (red) temperature isometric contraction. Inset: the A1 intensities obtained by subtraction of the relaxed profile from three other profiles.

Other estimates based on measurement of the intensities of the Bragg equatorial or meridional x-ray reflection vary significantly due to the dependence of the intensities of these reflections on lattice disorder [2]. Our figure is close to that derived from stiffness measurements in muscle fibres of the same type [5]. The data suggest that at 154 μM of myosin heads in permeabilized rabbit muscle fibres [6] and 300 kPa tension (Fig. 1) a myosin head strongly attached to actin during high temperature isometric contraction produces an average force of ~11 pN.

2. Structural changes in myosin motors caused by stretch of muscle fibres

In the second set of experiments we studied structural properties of myosin heads bound to actin in contracting muscle fibres during stretch at near physiological temperature. Stretch quickly increases instantaneous fibre stiffness [7]. It was suggested that stretch promotes binding to actin of the second partner head of a myosin molecule [8]. Although stretch is known to induce a significant decrease in the intensity of the M3 meridional x-ray reflection [8], changes in the layer line intensities caused by stretch were unknown. To reveal such changes at a near physiological temperature, bundles of muscle fibres were activated at low temperature and then subjected to a T-jump to 32-34°C. After a steady-state was achieved, the 2D x-ray diffraction pattern was collected for 30 ms and then the fibres were stretched at a constant velocity of ~1.2 muscle length/s. The second diffraction pattern was collected for 30 ms at the end of the stretch at near steady-state conditions. The filament sliding of ~20-25 nm per half-sarcomere at the beginning of the 2nd x-ray exposure was too large to allow bound heads to remain attached during the stretch, so that the measured stiffness and structural features were those of newly bound heads.

Apart from the known stretch-induced decrease in the intensity of the M3 meridional reflection we observed a decrease in the off-meridional intensities of the myosin M1, M2 and actin A1, A2 layer lines. We also observed a ~20% increase in fibre stiffness upon stretch as previously found in intact frog muscle fibres [7, 8]. The decrease in the myosin layer line intensities suggests a decrease in the fraction of detached myosin heads, thus supporting the notion of stretch-induced head

binding. On the other hand the significant decrease in the intensity of the actin layer line, especially A1, suggests that additional binding of myosin heads to actin is not accompanied by an increase in actin labelling by bound myosin heads. This can only be explained if the binding is non-stereo-specific, i.e. occurs over a range of azimuthal and possibly axial angles [1, 2, 4]. Thus the data suggest that a stretch promotes additional binding of myosin heads (possibly the partner heads) to actin although the binding is non-stereo-specific. Stretch also reverses the locking transition of myosin heads on actin so that the total fraction of bound heads increases while the fraction of stereo-specifically bound heads decreases [1]. The data explain low ATP consumption during stretch.

Two papers which describe the results of these experiments, “Duty ratio of myosin motor during isometric contraction of rabbit muscle fibers at near-physiological temperature” and “Stretch of contracting muscle promotes non-stereo-specific binding of myosin heads to actin” are in preparation and will be submitted to international journals in 2010.

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