Molecular mechanism of muscle contraction studied by X-ray diffraction in intact and demembranated fibres (SC-2782, Nov 09)

The aim of this project is to investigate the relation between the biochemical, mechanical and structural states of the molecular motor of muscle, the myosin head that cross-links the myosin and the actin filaments and generates force and shortening of muscle. This is possible extending the time-resolved X-ray diffraction approach to demembranated (skinned) fibres from mammalian muscle, in which the biochemical milieu can be controlled and the structural and mechanical changes in the myofilaments and myosin motors can be related to the level of activating Ca^{2+} and to the steps of the ATP hydrolysis cycle that fuels contraction. We have previously demonstrated during experimental visit SC-2051 that X-ray interference between the two halves of the sarcomere can be recorded at ID02 from horizontally mounted skinned fibres from rabbit psoas. In this visit we have investigated the temperature-dependence (range 4-20 °C) of the intensity and fine structure of the 2D X-ray diffraction pattern in the relaxed and in Ca2+ activated (pCa 4.5) fibres. The spatial resolution, signal:noise and sensitivity of the X-ray camera/detector system were significantly improved compared to previous experiments at ID02 by the combination of (1) the FReLoN CCD detector (active area 10 x10 cm^2, pixel size 52 µm, possibility of binning up to 8 pixels in the direction perpendicular to the fibre axis); (2) reduction of the horizontal width of the beam to 0.2 mm (FWHM) at an incident intensity of 10^{13} photons/s. In this way, the fine structure of the M3 reflection could be resolved in horizontally mounted fibres with a camera length of 5 m.

Fibre preparation and mechanical protocol. Small bundles (70-150 fibers) from the psoas muscles of rabbits are prepared in Florence, stored in relaxing solution containing 50% glycerol at –20 °C and transported to the ESRF in a refrigerator at -20 °C. For the experiments bundles of up to 3-4 fibres 5-6 mm long are dissected, their extremities are clamped with aluminium T-clips and horizontally mounted in a drop of relaxing solution between the lever of the motor and force transducer. Cycles of contraction-relaxation with preservation of sarcomere structure are obtained by using a solution exchange system combined with temperature jump (Linari *et al*. *Biophys J* 92:2476, 2007). The system at the beamline is implemented with a second stepper motor that moves the mechanical apparatus and fibre system down leaving the fibre enclosed in a narrow aluminium delimited air chamber with two windows for the X-ray path.

Results. Data reported are mean±SE from 3-7 bundles. Active isometric force was 176±30 kPa at 12°C ($T_{0,12}$), and 0.35 and 1.4 times $T_{0,12}$ at 4 and 20°C respectively. The lattice spacing $d_{1,1}$ at 4°C was 25.81±0.06 nm in relaxed fibers and 24.62±0.02 nm during activation and decreased with increase in temperature with a slope that was larger in activated fibers. The M3 meridional reflection from the axial repeat of the myosin heads was sampled by X-ray interference between the two halves of the myosin filament. During activation the intensity ratio of the two main M3 peaks (R_{M3}) varied from 0.37±0.07 at 4°C to 0.45±0.12 at 20°C. M3 spacing (S_{M3}) was 14.480±0.006 nm in relaxed fibers and increased on activation. Like force, S_{M3} in activated fibers increased with increasing temperature , and the S_{M3}-force relation had a slope of 0.320±0.026%/$T_{0,12}$ and a zero-force intercept of 14.510±0.004 nm, 0.21±0.05% higher than S_{M3} in relaxed fibers. The above results show that (*i*) filament lattice spacing reduces with active force, with a slope, corrected for the temperature effect in relaxed fibers, of -2.9±1.4%/$T_{0,12}$, (*ii*) the increase in myosin filament periodicity on activation is much smaller than the 1.5% reported for intact frog fibers (Linari *et al*. *PNAS* 97:7231, 2000) and (*iii*) the S_{M3}-force relation in the activated fibre indicates a myosin filament compliance of 6.40±0.52 nm/µm/$T_{0,12}$, similar to that in active frog fibers (Reconditi *et al*. *Nature* 28:578, 2004).

Perspectives. Based on the mass and quality of results achieved it is clear that we can expand the protocol to investigate, during the next visit, the Ca-sensitivity of the myofilament structural changes associated to activation. In this respect the skinned fibres provide the unique advantage that the changes occurring near the threshold for myosin motor attachment and force generation can be collected in steady state conditions changing pCa in the range 6-7, allowing longer exposure and thus with higher signal to noise ratio.

Publication from ESRF in the last 18 months

Full papers

E. Brunello, L. Fusi, M. Reconditi, M. Linari, P. Bianco, P. Panine, T. Narayanan, G. Piazzesi, V. Lombardi and M. Irving - Structural changes in myosin motors and filaments during relaxation of skeletal muscle. *J Physiol* **587**, 4509-4521, 2009.

Book Contributions

Brunello, E., Reconditi, M., Elangovan, R., Linari, M., Sun, Y.-B., Narayanan, T., Panine, P., Piazzesi, G., Irving and M., Lombardi, V. – The molecular basis of the braking action of muscle studied by X-ray interference. *ESRF Highlights 2008*, 53-54, 2008.

Abstracts

M. Linari, M. Reconditi, M. Caremani, E. Brunello, M. Dolfi, M. F. Martinez, T. Narayanan, G. Piazzesi, M. Irving, V. Lombardi - Changes in the low angle x-ray diffraction pattern from skinned fibers of rabbit psoas muscle on activation and force generation. *Biophysical Society Meeting Abstract. Biophys J, Supplement 1*, 2010

M. Reconditi, M. Linari, G. Piazzesi, M. Irving, V. Lombardi - Relative contribution of attached and detached myosin heads to the X-ray pattern from skeletal muscle. *Biophysical Society Meeting Abstract. Biophys J, Supplement 1*, 2010

E. Brunello, M. Reconditi, M. Linari, P. Bianco, T. Narayanan, P. Panine, G. Piazzesi, V. Lombardi, M. Irving. - Structural changes in the myosin motors during activation and force generation of muscle. *Biophysical Society Meeting Abstract. Biophys J, Supplement 1*, **96**: 212a/1097-Plat, 2009.

Brunello E, Linari M, Bianco P, Narayanan T, Panine P, Piazzesi G, Lombardi V, Irving M, Reconditi M. - Structural changes in the myosin motors during activation and force generation of muscle. *Acta Physiologica* **194**:19, OC13, 2008