Molecular mechanism of muscle contraction studied by x-ray diffraction in intact and demembranated fibres (SC-2989, Nov 10)

The aim of this project is to elucidate the molecular basis of muscle contraction and its regulation using combined mechanical and time-resolved X-ray diffraction experiments on demembranated fibres from mammalian muscle, where it is possible to control the biochemical milieu bathing the myofilaments. We have demonstrated during experiments SC-2051 and SC-2782 that x-ray interference between the two halves of the sarcomere can be recorded at ID02 from horizontally mounted demembranated fibres from rabbit. In this visit (SC-2989) we have investigated (i) the temperature-dependence (range 5°C up to the physiological temperature, 36°C) of the intensity and fine structure of the 2D x-ray diffraction pattern in the relaxed fibres and (ii) how the X-ray diffraction pattern of activated fibres depends on [Ca^{2+}] and [orthophosphate]. 20 ms X-ray frames were collected using a CCD FReLoN detector (active area 10x10 cm^2, pixel size 52µm, possibility of binning 8x in direction perpendicular to the fibre axis) and a 6m camera length. The longer camera length associated with a beam of 180 x 180 μm^2 (obtained via the slits) allowed a better spatial resolution compared with previous experiments (SC-2782).

Fibre preparation and mechanical protocol. Demembranated bundles of fibres from rabbit psoas muscles are prepared in Florence and stored and transported to the ESRF at -20°C in relaxing solution containing 50% glycerol. Before the experiments bundles of 3-5 fibres 5-6mm long are isolated, their extremities clamped with aluminium T-clips and horizontally mounted in a drop of relaxing solution between the lever of the motor and the force transducer. Cycles of contraction-relaxation with preservation of sarcomere structure are obtained by using the solution exchange system combined with temperature jump (Linari *et al.*, Biophys. J. 92:2476, 2007). The thermo-regulated trough has a Z movement that allows the plate carrying the solution drops to be lowered so that X-ray measurements can be made with the fibre in an air cavity in the centre of a temperature controlled aluminium block.

Results. The M3 meridional reflection from the axial repeat of the myosin heads was sampled by X-ray interference between half-sarcomeres. In relaxed fibres at 12°C, the M3 reflection had a major peak at 14.56 nm and a minor peak at 14.37 nm. The ratio of peak intensities (R_{M3}) was 0.43±0.06 and the spacing (S_{M3}) was 14.49±0.01 nm. The intensity of the main peak reduced with increasing temperature, so that at 36°C (the physiological temperature) the 14.37 nm peak was dominant, with small satellite peaks on either side, as in resting intact fibres from frog muscle. During activation at 12°C at saturating [Ca^{++}], pCa 4.5, the intensity of the M3 reflection (I_{M3}) increased to 1.9±0.4 times the relaxed value with major and minor peaks at 14.68 nm and 14.46 nm; R_{M3} was 0.62±0.03 and S_{M3} was 14.59±0.01 nm. Activation at pCa 5.5 or at pCa 4.5 with addition of 10 mM orthophosphate (Pi) had similar effects: force was reduced to 0.34±0.10 the control value and I_{M3} to 0.56±0.03; R_{M3} was 0.46±0.07 and S_{M3} was 14.55±0.02 nm. These results give structural support to the conclusion from mechanical experiments (Linari *et al*., 2007; Caremani *et al.*, Biophys. J. 95:5798, 2008) that both decreasing [Ca^{++}] and increasing [Pi] reduce isometric force by a decrease in the number of force generating myosin heads with no change in force per head.

Perspectives.

The sensitivity of the x-ray pattern to temperature is in agreement with previous work (Xu *et al.*, Biophys. J. 77:2665, 1999; Xu *et al.*, Biochemistry 42:390, 2003), but is in contrast with the absence of such effect on whole mammalian muscle at rest (Caremani *et al.*, Biophys. J. Suppl. 1, **100:**312a, 2011). The problem can be investigated by recording the effect of ionic strength and osmotic agents on the x-ray pattern of relaxed fibres.

Publication from ESRF in the last 18 months.

Full papers

1. S. Park-Holohan, M. Linari, M. Reconditi, L. Fusi, E. Brunello, M. Irving, M. Dolfi, V. Lombardi, T.G. West, N.A. Curtin, R.C. Woledge, G. Piazzesi, *Mechanics of myosin function in white muscle fibres of the dogfish Scyliorhinus canicula*, J. Physiol., in press (2012)
2. M. Reconditi*, E. Brunello*, M. Linari, P. Bianco, T. Narayanan, P. Panine, G. Piazzesi, V. Lombardi, M. Irving, *Motion of myosin head domains during activation and force development in skeletal muscle,* PNAS, 108 (17) 7236-7240 (2011); *equally contributed

Abstracts

1. E. Brunello, M. Caremani, M. Reconditi, M. Linari, M. Dolfi, M. Fernandez Martinez, T. Narayanan, G. Piazzesi, M. Irving, V. Lombardi, *The low angle X-ray diffraction pattern from skinned fibers of rabbit psoas muscle: effect of changes in [Ca^{++}] and [orthophosphate]*, Biophys. J., Suppl. 1, 102(3), 147a, (2012)
2. M. Reconditi, E. Brunello, M. Linari, L. Fusi, T. Narayanan, G. Piazzesi, V. Lombardi, M. Irving, *Structural changes in myosin heads and filaments during unloaded shortening and force redevelopment*, Biophys. J., Suppl. 1, 102(3), 147a, (2012)
3. G. Piazzesi, M. Reconditi, E. Brunello, L. Fusi, M. Linari, M. Fernandez, T. Narayanan, M. Irving, V. Lombardi, *Sarcomere-length dependence of the low angle X-ray pattern from skeletal muscle fibers at rest and during isometric contraction*, Biophys. J., Suppl. 1, 102(3) p. 147-148a, (2012)
4. E. Brunello, M. Reconditi, M. Linari, L. Fusi, T. Narayanan, G. Piazzesi, V. Lombardi, M. Irving, *Structural changes in myosin heads and filaments during unloaded shortening and force redevelopment after shortening*, Acta Physiologica, 203, Suppl. 688, 40, (2011)
5. M. Reconditi, E. Brunello, L. Fusi, M. Linari, M. Fernandez, T. Narayanan, M. Irving, V. Lombardi, G. Piazzesi, *Relative contribution of attached and detached myosin heads to the X-ray pattern determined in muscle fibres contracting at different sarcomere lengths*, Acta Physiologica, 203, Suppl. 688, 171, (2011)
6. M. Reconditi, M. Linari, G. Piazzesi, M. Irving, V. Lombardi, *A reinvestigation of the source of compliance of muscle cross-bridges*, *Biophys. J.,* Suppl. *1*, **100**(3), 585a, (2011)
7. G. Piazzesi, M. Linari, M. Reconditi, M. Caremani, E. Brunello, M. Dolfi, M. F. Martinez, T. Narayanan, M. Irving, V. Lombardi, *A low angle X-ray diffraction study of the relaxed and activated states of skinned fibres of rabbit psoas muscle*, Acta Physiologica, 200, Suppl. 681, 172, (2010)
8. M. Reconditi, M. Linari, G. Piazzesi, M. Irving, V. Lombardi, *X-ray diffraction estimate of the relative contributions of subfragment-1 and -2 of the myosin molecule to the compliance of muscle cross-bridges*, Acta Physiologica, 200, Suppl. 681, 173, (2010)

ESRF website
http://www.esrf.eu/news/general/muscle-contraction/index.html

University of Florence (Italy) website
http://www.unifi.it/mod-MDNotizie-master-action-view-bid-3002.html