Experimental Report
Proposal SC-2662
Structure-function relation in skeletal muscle
15 shifts, 15-20 April 2009

The widely accepted model of muscle contraction proposes that the globular part of the myosin molecule forms cross-bridges between the myosin and actin filament and generates force through an ATP-driven working stroke. The working stroke was originally thought to stress a compliance in the cross bridge itself. Since the middle 1990's a large body of experimental evidence has been accumulated which indicates that both actin and myosin filaments considerably contribute to the half-sarcomere compliance, but there is still debate about the value of filament compliance in different mechanical conditions: X-ray diffraction experiments on whole muscle and muscle fibres have shown that the filament compliance is not the only responsible of filament elongation, but also that structural rearrangements occur that change the periodicities along the filaments and depend both on activation and on the level of force. While the changes in the periodicity of myosin filament at various levels of force between 0 and T_0 (the maximum force attained by the muscle under isometric contraction) have been investigated (Huxley et al. 2006; Piazzesi et al. 2007), the changes in the periodicity of the actin filament in the active muscle have been reported only at 0 force and at T_0 (Bordas et al. 1999).

The main aim of the experiments performed during the SC-2662 visit was to investigate the changes in the periodicities along both actin and myosin filament in the same preparation at different levels of force, modulated with ramp stretches and releases applied to contracting muscles of frog (*Rana esculenta*). In particular, we wanted to determine the spacing changes of the actin filament in the active muscle in the unexplored region of forces between 0 and T_0, to determine what is the relative contribution of compliance and of structural rearrangements to the elongation of the myofilaments.

Methods.

Whole sartorius muscles, ca 3 cm long, were dissected from *Rana esculenta* and mounted vertical in a thermo-regulated chamber containing oxygenated Ringer's solution (4°C) at the beamline. The muscle length L_0 was adjusted to be in the plateau region of the force-length relation with a micromanipulator clamped to the pelvic bone. A muscle lever system type 300C-LR (Aurora Scientific Inc.) interfaced with a PC-based system for control and acquisition (National Instruments) controlled the muscle length and recorded force.

Mechanical protocol. The muscles were activated through electrical pulses (25 Hz) to obtain a fused tetanus. Once the tetanic force (T_0) was fully developed, the muscles were allowed to shorten at different velocities (0.5-1.6 L_0/s), to reach steady forces in the range 0.5 – 0 T_0. Shortening was stopped when muscle length was reduced by 8%. A slow (0.1 L_0/s) ramp stretch was also imposed on the top of the tetanic plateau to reach a force of ca 1.5 T_0 when muscle was stretched by ca 2% L_0.

X-ray diffraction. At the beamline X-ray wavelength was 0.1 nm and the flux on the sample was about $2 \cdot 10^{13}$ ph/s. Diffraction patterns were collected in 25ms exposure windows at rest, at T_0 and during the last part of the ramps. Exposure windows were controlled with two fast electromagnetic shutters (< 1ms opening time). The patterns were acquired with the Kodak CCD-based detector available at the beam line (FReLoN, active area 10x10 cm^2, 2048x2048 pixels). The pixels were binned 8x1 (HxV) to reduce the readout noise while keeping high the resolution along the meridional axis. Binning also allowed to reduce the detector dead time between successive acquisitions to ca 100ms and thus to collect the patterns at the three different states during the same contraction. The detector-specimen distance was set to 1.2m to collect data up to 0.4 nm^{-1} in the reciprocal space. Between tetani, the specimen was axially shifted to spread and reduce radiation damage.

*Data analysis.*Mechanical data were analyzed with a software developed in the LabView (National Instruments) environment. 2D patterns were analyzed with Fit2D (A. Hammersley, ESRF). The

intensity in the 4 quadrants of the patterns was averaged to increase the signal-to-noise. The 1D intensity distribution was obtained by averaging the intensity between 0.02 nm^{-1} on either side of the meridional axis for the meridional reflections and in the range 0.035-0.06 nm^{-1} for the actin-based layer lines. 1D intensity distribution was background subtracted with a convex hull algorithm. The spacings of the reflections was measured with SigmaPlot (Systat Software Inc.) as the centre of mass of their intensity distribution. When the reflection of interest was clearly overlapped with another reflection, Gaussian fit was used to separate the intensity peaks.

Results

Myosin-based reflections. The periodicity changes along the myosin filament were evaluated by the position on the meridional axis of the myosin-based M6, M9 and M15 reflections at 7.2, 4.8 and 2.9 nm respectively, which are thought to bring information mainly on the backbone of the thick filament and to be little influenced by the myosin cross-bridges. Both the M6 and M15 peaks move toward the centre of the pattern by ca 1.25% from rest to T_0, which indicates a corresponding increase in the axial periodicity. This spacing change is about 5 times higher than what is expected from the compliance of the myosin filament (Huxley et al. 1994; Wakabayashi et al. 1994; Reconditi et al. 2004) and has been attributed to structural changes that occur in the filament upon activation and force development (Huxley and Brown 1967; Brunello et al. 2006). The M9 reflection is too weak at rest and its spacing in that condition has not been measured. When force is modulated with ramp stretch or releases, the spacings of the reflections first decrease with force with a slope 0.13-0.19 %/T_0 in the range 0.45-1.6 T_0, and then decrease with a slope 2-2.5 times steeper, recovering almost half-way toward the rest periodicity at unloaded shortening (Piazzesi et al. 2007).

Actin-based reflections. The periodicity changes along the actin filament were evaluated by the position on the meridional axis of the actin-based A13 and of the ALL6 and ALL7 layer lines at 2.7, 5.9 and 5.1 nm respectively. The periodicity of the actin filament increases by ca 0.20% from rest to T_0, a value close to that previously reported by structural studies (Huxley et al. 1994; Wakabayashi et al. 1994; Bordas et al. 1999). When force is modulated with ramp stretch or releases, the spacings of the reflections first decrease with force with a slope 0.15-0.20 %/T_0 in the range 0.45-1.6 T_0, and then decrease with a higher slope, with a ca 0.4% total change between V_{max} and T_0, that brings the spacings below their value at rest. The change of the actin filament periodicity with force in the range 0.45-1.6 T_0 is close to the change from rest to T_0 and consistent with the value of actin compliance determined with mechanical experiments in sigle fibres on the same species used here (Linari et al. 1998). These findings suggest that in active muscle, at forces below 0.4 T_0, structural changes must occur both in the myosin and actin filament.

A preliminary report on these results has been presented at the annual meeting of the Physiological Society of Italy, Siena (Italy), 23-25 September 2009 (L. Melli, M. Caremani, M. Dolfi, M. Fernandez-Martinez, T. Narayanan, M. Reconditi. - Changes in periodicities of actin and myosin filaments at different levels of force. *Acta Physiologica* 197, Supplement 672:P105, 2009).

References

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