STRUCTURAL CHANGES IN MYOSIN ACCOMPANYING FORCE DEVELOPMENT AND STEADY SHORTENING IN SINGLE INTACT FIBRES FROM FROG SKELETAL MUSCLE

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Generation of force and shortening in a contracting muscle are attributed to the interaction of myosin cross-bridges extending from the thick filament and the thin actin filament. We investigated the underlying structural changes in intact fibres from frog skeletal muscle (Rana temporaria) by time resolved X-ray diffraction, using the intense highly collimated beam at ESRF, Grenoble, beamline 4. X-ray data were collected in 10 ms time frames with a 2D gas filled detector during the rise of the tetanic tension and the subsequent steady shortening at a velocity (0.5 fibre length/s) that gives nearly the maximum power output. Changes in intensity and spacing of the third-order myosin meridional reflection, arising from the axial mass projection of the myosin heads, could be precisely measured by signal averaging from only one fibre. Fibres were mounted between a force and a length transducer by means of aluminium foil clips to minimise the series compliance. Tension development (half-time 33 ms, at 4 C and 2.2 um sarcomere length) is accompanied by an increase in axial spacing of the reflection from 14.34 nm (rest value) to 14.57 nm (tetanus plateau value, Huxley & Brown, J. Mol. Biol., 1967, 30, 383), which has been attributed to myosin heads attaching to actin (Bordas et al., J. Muscle Res. Cell Motility, 1993, 14, 311). The reflection intensity decreases to 50% of the resting value within 30 ms, due to the loss of resting crystallographic order (Huxley et al., J. Mol. Biol., 1982, 158, 673), then increases with a time course that almost superimposes tension development. Steady shortening decreases the tension and the intensity of the reflection to 60% and 50% of their respective isometric values, but does not affect the spacing of the reflection by more than 0.3%, indicating that the intensity signal arises from myosin cross-bridges interacting with actin. Consistent with previous work (Huxley et al., J. Mol. Biol., 1983, 169, 469; Irving et al., Nature, 1992, 357, 156; Piazzesi et al., Biophys. J., 1995, 68, 92s), these results suggest that attached myosin heads developing isometric force have their long axis nearly perpendicular to filament axis, while during the execution of the working stroke that drives active shortening they undergo a change which broadens their axial mass projection.