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Static and dynamic structural properties of the pre force-generating myosin heads in contracting mammalian muscle fibres

Objectives
The aim of the experiments was to study structural changes in the actin-myosin molecular nano-motor responsible for force generation in muscle. Changes in configuration and mode of attachment of myosin heads to actin were studied during shortening at a constant load and during a stretch of contracting muscle fibres using 2D low angle x-ray diffraction. In particular we tried to distinguish two different types of structural transitions: tilting of the light chain domain ('lever arm') of myosin head with respect to its catalytic domain and the 'roll and lock' transition of the whole head from a non-stereo-specifically to a stereo-specifically bound state and to study the strain- and load-dependence of these transitions.

Results
In two sets of experiments, mechanical and structural changes in bundles of three permeabilized muscle fibres from rabbit psoas muscle were studies after transition from isometric contraction to periods of shortening or stretching under a constant force. Muscle fibers were partially cross-linked with EDC to protect their structural homogeneity, activated at low temperature and then heated to near physiological temperature using joule T-jump technique. X-ray diffraction patterns were collected in near-isometric conditions and during 2 ms long contractions under load of 20-30% of T_0 or 140-160% T_0 where T_0 is isometric tension. During shortening instantaneous muscle stiffness decreased by ~30%. The intensities of all actin layer lines in the diffraction pattern also decreased by 10-30%. The intensity of the actin-myosin beating layer line at 10.3 nm dropped by 80% demonstrating that a tilt of the 'lever arms' of a majority of myosin heads is facilitated by a decrease in load. Stretches induced an increase in stiffness without significant changes in the intensities of all actin and actin-myosin layer lines except first actin layer line at 36 nm. This observation shows that a stretch initiates a reverse in the 'roll and lock' transition of myosin heads bound to actin as predicted by our recent model of structural changes responsible for force generation in muscle (Ferenczi et al., 2005). In total 20 experiments have been performed during the beam allocation period and the diffraction patterns from 900 and 1100 repeats of the 1st and 2nd experimental protocols, respectively, were collected and added together so that sufficient signal-to-noise ratio was achieved for reliable measurement of changes in intensity and position of the relatively weak and partially overlapping layer lines. In control experiments, the effect of EDC cross-linking on the structural properties of myosin heads in muscle fibres was assessed and found to be negligible.

Recent relevant publications.
Tsaturyan A.K., Koubassova N, Ferenczi M.A., Narayanan T., Roessle M. and Bershitsky S.Y. (2005) Strong Binding of Myosin Heads Stretches and Twists the Actin Helix. *Biophysical Journal* **88(3)**:1902-1910
Ferenczi M.A., Bershitsky S.Y., Koubassova N., Siththanandan V., Helsby W.I., Panine P., Roessle M., Narayanan T., Tsaturyan A.K. (2005) The 'roll and lock' mechanism of force generation in muscle *Structure* **13**: 131-141