Structure-function relation of the myosin motor in striated muscle

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The aim of our research is to elucidate the mechanism of energy transduction by the molecular motor in muscle (Fig. 1).

The working stroke responsible for force generation and interfilamentary sliding is due to a structural change in the globular part of the myosin molecule (the myosin head) cross-linking the myosin and the actin filaments. Both the size of the working stroke and its biochemical, energetic and isotonic features remain controversial.

We use single muscle fibre mechanics and time-resolved interference X-ray diffraction to study the myosin motor in situ with sub-nanometer resolution. Our present activity is dedicated to clarify the following problems:

1) the size of the myosin working stroke and its dependence on the load;
2) the conformational change in the myosin head leading to isometric force generation;
3) structural events in the myofilaments during activities of contraction.

The answers to these questions are essential for relating molecular and cellular studies of myosin motors, and for elucidating the mechanisms of efficient mechano-chemical coupling.

METHODS

Single fibres from the tibialis anterior muscle of Rana temporaria of 2.2 pm sarcomere length are vertically mounted in a trough containing physiological solution between a force transducer and a loudspeaker coil motor (Fig. 2). Patterns are collected on the imaging plate detector (IP) or on the image intensified FastLab CCD detector placed at either 30 or 4 m. The intensity and linear divergence of the beam at ID22 baseline allow to resolve the resolution of structural studies of the muscle motor by exploiting X-ray interference from the two arrays of myosin heads in each thick filament (Fig. 3).

RESULTS

1. The size of the working stroke and its dependence on the load


c) With a fast force clamp method we could determine the working stroke elicited by the drop in force to different fractions of the isometric force (Experiments conducted both at ID22, ESRF, and at BicAT, APS).

The working stroke responsible for force generation is an endothermic process (Piazzesi et al., J. Physiol. 519, 99-106, 2003). Filament sliding is exothermic. Are the two processes driven by the same structural transition? We record the axial movement of the myosin heads associated to changes in the isometric force changes in temperature in the range 0-17°C.

2. The structural change leading to isometric force generation is an endothermic process (Piazzesi et al., J. Physiol. 519, 99-106, 2003), filament sliding is exothermic. Are the two processes driven by the same structural transition? We record the axial movement of the myosin heads associated to changes in the isometric force changes in temperature in the range 0-17°C.

3. Changes in the thick filament at the start of contraction

i) Time course of attachment of force generating myosin heads during the tetanus rise.

Changes in spacing of the M3 reflection during the rise of the isometric tetanus (T&T) and the effect of superposing a ramp shortening (R5) at a velocity that prevents the force rise (5 ms time frames collected with the FAST detector).

ii) Structural changes in the myosin heads during the development of the isometric tetanus

The X-ray interference technique will be applied to determine the structural transition in the myosin heads that is coupled to the rise in filament strain during the development of the tetanus.


Fig. 2 Scheme of the setup at the baseline

Fig. 3 X-ray interference between the two bipolar arrays of myosin heads allows to measure the linear motion of myosin heads that drive the actin filaments toward the center of the sarcomere. In the isometric contraction (T0) the peak intensity ratio (R=IHA/ILA) is 0.8. A step forward in the working stroke can be entropically driven.

Fig. 1