
Non-Processive Molecular Motors on a Leash: a Novel Single-Molecule, Microsecond Resolution Force Clamp

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Myosin II is the motor protein that drives muscle contraction through cyclical interactions with an actin filament. In each cycle a single ATP molecule is split and a filament displacement (or working stroke) is generated. The working stroke produced by a single myosin head has been previously measured in isolated myosin molecules, but the effects of the high loads acting on the myosin molecule during muscle contraction could not be investigated. In fact, current single molecule techniques apply force with a delay of few milliseconds after actin-myosin binding, when the working stroke of skeletal muscle myosin has already been completed.

Here, we developed a novel single molecule technique in which a constant force is continuously applied to the actin filament, so that the delay between myosin binding and force application is abolished. This method is capable of resolving the development of the myosin working stroke under different loads with a very high time resolution and detecting events as short as 100 μ s due to a very high signal-to-noise ratio.

We found that under loads in the range 1 to 10 pN myosin can follow two distinct pathways in its interaction with actin: a population of very fast events ($240 \pm 23 \mu$ s) in which myosin detaches from actin before producing any movement (the duration of these events does not depend on ATP concentration), and a second population of events where myosin steps and remains bound to actin for a longer time. For low forces ($|F| < 2$ pN) the lifetime of this second population of events linearly decreases with ATP concentration in the range 5-50 μ M. The mean amplitude of the myosin working stroke is found to be smaller at increasing loads and vanishes at the isometric force (5.7 ± 0.6 pN). On the other hand, the rise time of the working stroke becomes longer as the force increases.