Harmonic Force Spectroscopy Reveals a Force-Velocity Curve from a Single Human Beta Cardiac Myosin Motor

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Harmonic Force Spectroscopy Reveals a Force-Velocity Curve from a Single Human Beta Cardiac Myosin Motor
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In vitro motility assays with surface-adsorbed actomyosin motor fragments have given important insights into the molecular physiology and pathology of striated muscle contraction and inspired nanotechnological applications e.g. lab-on-a-chip devices. However, to date neither precise localized control of the motor density nor introduction of nanoscale three-dimensional geometrical constraints has been possible. This hampers studies of cooperative phenomena and realization of three-dimensional (3D) transport systems. Here, we take critical steps to overcome these limitations, using aluminium oxide coated gallium phosphide nanowires as scaffolds for heavy meromyosin (HMM) adsorption. The wires (diameter: 100-200 nm; height < 5 µm) were either positioned vertically or horizontally on a metallic surface to give hollow nanowires. Upon ATP addition, actin filaments were propelled by HMM on top of the arrays, with filaments spanning inter-wise distances up to 1 µm. The filaments also moved up and down vertical nanowires as detected using sub-wavelength light guiding properties of the nanowires. Motility on top of nanowire arrays holds potential for studies of cooperative phenomena e.g. local enhancement of myosin binding along actin filaments upon actomyosin interactions. Here we tested whether the low velocity for long filaments seen at uniformly low motor densities on flat surfaces may be attributed to loss of cooperative enhancement of myosin head binding locally to actin close to an existing actomyosin cross-bridge. Velocity data showing 1/3 the velocity with 1 µm compared to 300 nm inter-wise spacing, in both cases ~90 HMM molecules per 150 nm wide wire tip (giving high local motor density), argue against this idea. We further demonstrate HMM propelled transport through hollow nanowires of 80 nm inner diameter. Uses of hollow nanowires in fundamental studies of actomyosin and in nanotechnological applications will be considered.

The Minimal Group Size for Globally Coordinated Stepping of Muscle Myosins Depends on ATP Hydrolysis Free Energy
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In motility assays of muscle myosins, a distinct motile state emerges with increasing number of mechanically coupled myosin binding sites (N). This is explicable by coordinated myosin stepping (CMS): build-up of pre power stroke (PS) myosins is followed by a whole group PS and detachment cascade [1]. At low N, singular infrequent detachment cascades occur; at intermediate N, cascades group into bursts; for high N, interruptions between bursts disappear [1]. Here, we investigate how changing ATP hydrolysis free energy (ΔG) affects N-dependent emergence of CMS. We extract motility assays of muscle myosins and changed P0 concentration ([P0]=0.1,2.5,5.15,30 mM, ionic strength adjusted by [KCl], [ATP]=2 mM, [ADP]=0.2 mM). Resolving actin sliding velocities by N [2] showed that increasing [P0] increased the N at which bursts and continuous filament motion emerge. Lowering the rate of P0 release reproduced these observations in our detailed mechanochemical model of linearly elastic myosins mechanically coupled via an actin filament [1]. In this model, the rate of myosins’ mechanical steps increases monotonically by ~6 orders of magnitude dependent on the skew in myosin cross-bridge strains (S). Plotting S and the fraction of myosin in the pre and the post PS state (n3,n2) reveals globally coordinated behavior that changes with N. N<5: a quiescent state with high n3 dominates; N>15: a quiescent state and cascading behavior with lowered n3 alternate; N>30: cascading behavior dominates. An according continuous model shows two N-dependent steady states representing quiescence and cascading. Adding stochastic fluctuations in S lead to N-dependent cascade-like cycles. This suggests global coordination of myosins, which occurs above a minimal myosin group size that depends on ΔG.


Toward the Realization of a Sarcomere-Like Machine
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We report the progress toward the realization of a synthetic sarcomere-like machine consisting of an array of motor proteins, regularly distributed on an inorganic nano-structured surface, interacting with a single actin filament. The actin filament, in its turn, is coupled to the output of a Dual Laser Optical Tweezers system (DLOT, range 0.5-200 pN force and 1-10,000 nm displacement) under either nano-positioner control or force control (Bianco et al. Biophys. J. 101:866-874, 2011). The correct polarity of the actin filament (5-15 µm long) is controlled by attaching its barbed end to a trapped bead via gelsolin (Suzuki et al. Biophys. J., 70:401-408, 1996). Mechanical measurements have been carried out with a simplified version of the machine in which the motor proteins (HMM from skeletal muscle of frog or rabbit) are randomly adsorbed on the flat tip of an etched optical fiber (diameter 4 µm), the position of which is controlled by a piezoelectric nano-positioner. In ATP-free solution the rupture force of the single actin-HMM bond (nano-positioner control) is 12.85 ± 0.35 pN. The bond lifetime under a load of 8 pN (force control) has a bi-exponential distribution and the time constant of the longer component is 1 s. The whole assembly in rigor at slack length is exposed to a solution with 2 mM ATP, force develops up to a steady value of 50 pN with a rise time of 2 s, more than one order of magnitude lower than the ATP-free state.