Investigation of Molecular Level Stress-Strain Relationships in Systems of Entangled F-actin by Combined Force-Measuring Optical Tweezers and Fluorescence Microscope

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Abstract

Actin plays a major role in cell structure, cell motility, vesicle and organelle transport, and muscle contraction. Actin’s ability to play these major roles is a direct consequence of the intricate relationship between stress and strain in a variety of filamentous actin (F-actin) networks. A thorough understanding of the unique stress-strain relationships in complex F-actin networks at the molecular level is currently lacking despite the importance of such networks to fields such as biometric material engineering and cell biology. Here we develop novel single-molecule instrumentation that combines dual-trap force-measuring optical tweezers with fluorescence microscopy to enable simultaneous characterization of intramolecular forces and molecular dynamics within F-actin networks. This instrumentation is combined with a novel technique in which single F-actin “probes” are used to apply molecular-level strains and measure induced stress within entangled F-actin systems while the deformations and dynamics of surrounding fluorescent labeled filaments are simultaneously imaged. Specifically, a fluorescent-labeled, microsphere-conjugated probe filament is held by its ends via dual optical traps, and the force induced on the probe is measured as it is moved through a network of selectively labeled entangled F-actin by using a nanoposition piezoelectric stage to move the sample chamber relative to the traps. Fluorescence microscopy is used simultaneously to image the dynamics of the labeled probe as well as the surrounding labeled molecules subject to deformation. Bioconjugation of the fluorescent polystyrene microspheres to the ends of labeled F-actin is achieved by carbodiimide attachment of biotin to microspheres and combining gelatin-coated microspheres with fluorescent-labeled actin. This powerful single-molecule technique allows simultaneous measurement of intramolecular forces and deformations of single molecules, providing the much needed link between stress and strain at the molecular level in complex F-actin networks.

Instrumentation

High Speed Prolonged Imaging

To enable simultaneous measurement of stress-strain dynamics, labeled probes are held by dual optical traps and their displacement is measured using a piezo-electric stage. The measured force is compared with the deformation visualized to obtain a relationship between stress and strain in a variety of filamentous actin (F-actin) networks. The measured force is compared with the deformation visualized to obtain a relationship between stress and strain in a variety of filamentous actin (F-actin) networks.

Force Detection and Calibration

Because optical traps behave similar to a spring, the force exerted on a trapped object can be described with Hooke’s Law: F = -kx, where k is the stiffness of the trap and x is the displacement. Meanwhile, the trap deformation, and the time are recorded at a rate of 1 kHz using Labview, a graph processing and data analysis system. To relate both the x-direction (top) and y-direction (bottom), both stage velocities are reduced by a factor of 10 to make the scales of velocity and laser deflection comparable. The average trap constants (using 10 trials of 10 seconds each for each axis) are 3.2 ± 0.01 pN/μm for kx and 2.1 ± 0.01 pN/μm for ky respectively. The two trap constants are very similar indicating the symmetry of the trap. The calibration for the trap. To use this method, we trap a microsphere (2 μm in diameter, in a 3% polyacrylamide gel containing 0.05% Triton X-100) with a single laser (488 nm) at a rate of 5 kHz. The stage velocity (and thus the velocity of the trapped microsphere) is calculated by differentiating the stage position with respect to time. The stage velocity and measured PSD signal are then recorded versus time for both the x-direction (top) and y-direction (bottom). Both stage velocities are reduced by a factor of 10 to make the scales of velocity and laser deflection comparable. The average trap constants (using 10 trials of 10 seconds each for each axis) are 3.2 ± 0.01 pN/μm for kx and 2.1 ± 0.01 pN/μm for ky respectively. The two trap constants are very similar indicating the symmetry of the trap.

References