Nanomechanical Properties of Motor Proteins

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Abstract:

Motor proteins drive essential processes in the body, including muscle movement and cell division, by converting chemical energy to mechanical work. Yet, the relationship between the structure and function of these molecular motors is not well understood. Acquiring comprehensive knowledge of these interactions could ultimately lead to the creation of nanoscale proteins with tunable properties. Such proteins could vastly improve cancer and disease research as well as provide fundamental insight into cell biology. The present study aims to further investigate nanomechanical properties of kinesin, a motor protein responsible for intracellular transport. To accomplish this, we successfully generated a recombinant human kinesin construct, labeled with green fluorescent protein and a histidine epitope tag, and used high-resolution imaging to characterize its velocity and photostability *in vitro*.

Introduction:

Kinesins are one group of motor proteins that transport cargo and play a critical role in cell division [1]. In cells, kinesin molecules attach to, "walk" along, and detach from microtubules, one type of cytoskeletal polymer, by a series of chemical reactions involving adenosine triphosphate (ATP) [2,3]. Kinesin proteins can be expressed in bacteria cells and characterized *in vitro*. Observing this walking process in reconstituted systems can help to better understand the relationship between kinesin and microtubules, and will provide insight into the motor mechanism of kinesin.

One challenge for *in vitro* measurements of motor proteins is collecting sufficiently high signals to allow for visualization. Total internal reflection fluorescence microscopy (TIRFM) provides a useful solution. In TIRFM, a laser beam is steered into a microscope objective at a high incident angle. Above a critical angle, the majority of the incident light is reflected, but a small portion propagates into the sample, parallel to the surface, as an evanescent wavefront that excites fluorophores on and near the surface [4]. This decreases the fluorescence emission from within the sample and increases the signal to noise ratio. By using two lasers of distinct wavelengths, kinesin and microtubules can be distinguished if labeled with two different fluorescent dyes that possess excellent photostability characteristics. The present study used an electron-multiplying charged coupled device (EMCCD) camera with the TIRFM system to provide fast, high-resolution data acquisition in order to characterize the velocity and photo-properties of fluorescent kinesin proteins.

Experimental Procedure:

A protein expression protocol was developed and used to generate recombinant kinesin proteins. *Escherichia coli* bacteria, containing a plasmid with the *kif5b* kinesin gene fused to green fluorescent protein (GFP) for visualization and a histidine tag for purification, were obtained and replicated. This multi day procedure required sterility and ideal temperature and chemical conditions. After growth, cells were lysed by sonication, releasing cellular contents into solution. Kinesin was column purified using a type of nickel resin, which had high affinity for the histidine-tagged kinesin. Expression products were analyzed with gel electrophoresis.

Gliding filament assays were used to characterize the recombinant kinesin. Coverglasses were coated with GFP antibodies to bind kinesin to the surface. Because the GFP is located at the tail, each motor head pointed up into solution and attached and detached to microtubules in solution, thus causing them to glide. A 532 nm laser and a custom TIRFM system were used to visualize microtubules labeled with rhodamine dye.

For each microtubule, 200 frames were collected at a frequency of 10 frames per second with an EMCCD camera. Analysis software was implemented to locate the coordinates of the tip of each microtubule in the first and last frame (Figure 1). The distance between these points was calculated and divided by the elapsed time (20 seconds) to obtain velocity data. To assess photostability, single kinesin-GFP molecules were observed using a 473 nm laser.

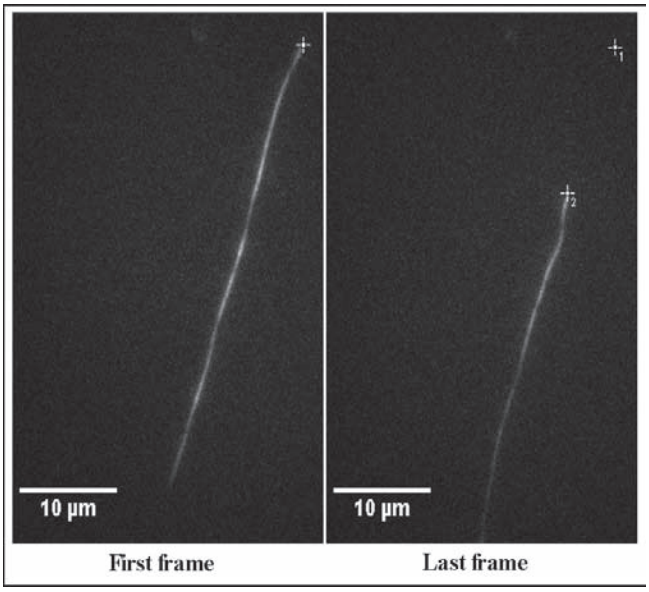

Figure 1: Analysis of a microtubule gliding assay video.

Results and Conclusions:

Green protein fractions and gel electrophoresis confirmed that GFP-fused kinesin was successfully made. To assess the functionality, microtubule velocities were calculated via gliding assays. The velocities of 160 microtubules were measured to be 650 ± 80 nm/s, consistent with earlier reports [5,6]. The distribution (Figure 2) was slightly skewed toward lower velocities, perhaps indicating the presence of non-or partly-functional motor proteins or imperfect surface immobilization.

Kinesin motors were also visualized at the single molecule level, and the fluorescence intensity was measured as a function of time to assess dye lifetime and brightness. The lifetime of a single GFP molecule was found to be ~ 10 seconds, as indicated by the steep vertical drop in mean intensity (Figure 3). This singular drop confirmed that single molecule signal detection had been achieved.

Future Work:

The robust protocol for the expression and characterization of kinesin motors that was developed will enable future analysis of a wide variety of motor proteins. In order to further improve the procedure, optimal conditions for single molecule imaging to reduce photobleaching, without impairing kinesin's functionality, should be investigated. Significant insight into the biology of motor proteins will be gained, which will help develop the promising concept of making proteins with desirable properties for cancer and disease applications.

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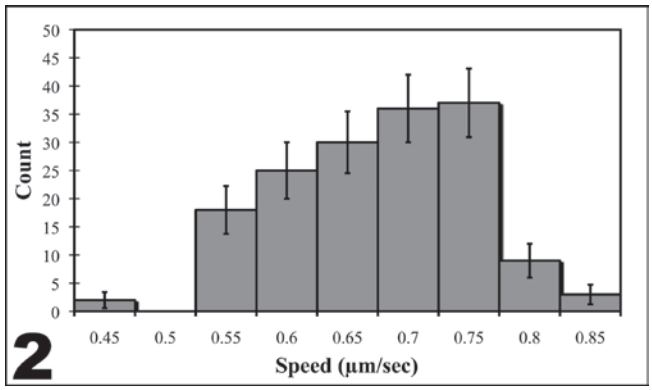

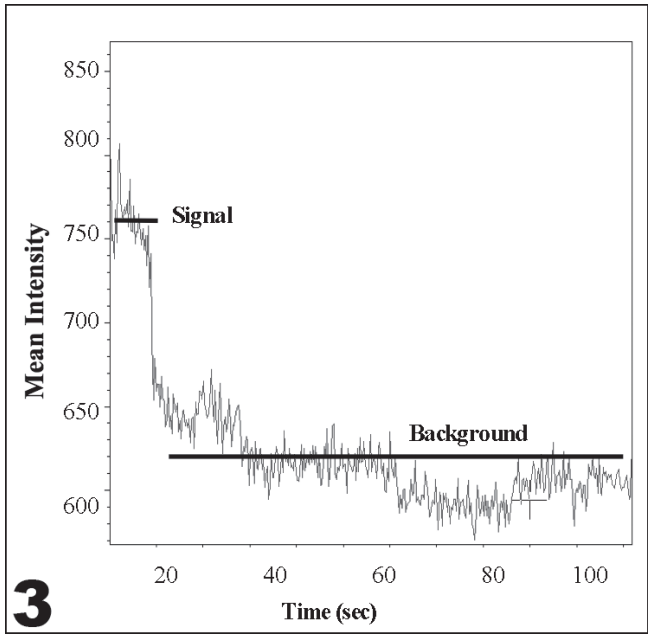

Figure 2, top: Distribution of microtubule speeds on kinesin-coated surfaces.

Figure 3, bottom: Photobleaching trace of a single GFP kinesin.