NANO
LETTERS

XXXX
Vol. xx, No. x
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Velocity Modulation of Microtubules in Electric Fields

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Received June 26, 2008; Revised Manuscript Received October 16, 2008

ABSTRACT

We show that the speed of microtubules gliding over a kinesin-coated surface can be controlled over a wide range of values by the application of an electric field. The speed can be increased by up to a factor of 5 compared to the speed at zero field when assisting forces are applied and slowed down to zero velocity for opposing fields. Sideways applied fields also induce significant motion. The kinesin surface density impacts the rate of velocity change, whereas the ATP concentration does not seem to play a major role, provided that it is nonzero. A simple grab-and-release model is presented that explains the velocity change with applied electric fields.

Kinesins are molecular motor proteins that move along microtubules in 8 nm steps with run lengths of about 100 steps, where one ATP molecule is hydrolyzed at each step.[1] Inverted gliding assays,[2] where fluorescently labeled microtubules are moved on top of a kinesin-coated surface, have been used to study various properties of these systems.[3−5] Gliding assays provide fundamental information not only on processively translocating motors such as kinesin but also on nonprocessive motors such as myosin.[5] Investigations of the interaction between the motors and the filaments under applied external forces can provide a better understanding of the binding and release rates of the motors. The capability to control motion and steering also opens the way toward a nanotechnological use of these carriers, where microtubules can be used as cargo shuttles between separated areas on a microfluidic chip.

Here, we present gliding experiments performed in kinesin-coated fluidic channels under different applied electric fields. Previous measurements[6] have demonstrated the versatility of these channels, allowing the confinement and steering of gliding microtubules and the possibility to apply large directional electric fields. The electric fields induce uniform applied forces that lie within the plane of movement of the microtubule, which provides an advantage over other force-applying techniques such as optical tweezers, where significant out-of-plane components are present.[7,8]

We observe a marked dependence on the velocity of the gliding microtubules with the applied field. Under assisting forces, that is, when the force is along the direction of movement of the gliding microtubule, we observe a very large increase of the microtubule gliding speed of up to five times the zero-field velocity. Under opposing forces, gliding velocities are reduced and the motion can even be stalled. While our results for opposing forces are similar to previously published work,[9,10] where kinesins are stalled for opposing forces larger than 7 pN, we observe a marked velocity increase under assisting forces. This is unexpected and not previously observed. We discuss various models for the observed behavior and suggest a mechanism of grab and release.

Figure 1a displays the scheme of the experimental setup. Microfabricated fluidic channels are coated with the help of pressure-driven flows of respectively casein and kinesin solutions to constitute a kinesin-coated surface on the channel. Subsequently, fluorescently labeled paclitaxol-stabilized associated-protein-free microtubules are added (as described before, see ref 6). Fluorescence microscopy allows us to accurately follow the microtubules as they glide along the surfaces. Experiments were done in a Pipes 80 mM buffer with 10 mM ATP, unless stated differently. Electric fields are generated by applying a voltage difference along the channel by use of two platinum electrodes placed in reservoirs that are spatially far removed from the imaging area. Snapshots at 1 s intervals allow measuring the gliding velocities. Only microtubules whose trajectories lie parallel or perpendicular to the electric field to within 5° are selected for quantitative analysis. Microtubules moving parallel to the fields fall into two categories: those in which the force is assisting the motion and those where the force opposes the gliding motion.

Figure 1b displays equally time spaced snapshots of gliding microtubules under parallel electric fields, from −100 V to +200 V over the 400 μm length of the channel. In

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10.1021/nl801837j CCC: $40.75
Published on Web 10/28/2008

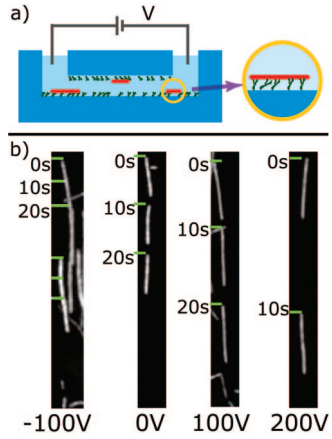

Figure 1. (a) Schematic representation of the measurement setup. The surface of a microfluidic channel (light blue) is covered with kinesins (green). Microtubules (red) glide on top of the kinesins. A voltage is applied by the use of electrodes placed in the reservoirs at opposite ends of the channel. (b) Superposed snapshots of gliding microtubules under different electric fields. From left to right: −100 V, 0 V, 100 V, and 200 V. At negative voltages (assisting force) the gliding microtubules speed up, whereas they slow down for positive voltages (opposing force).

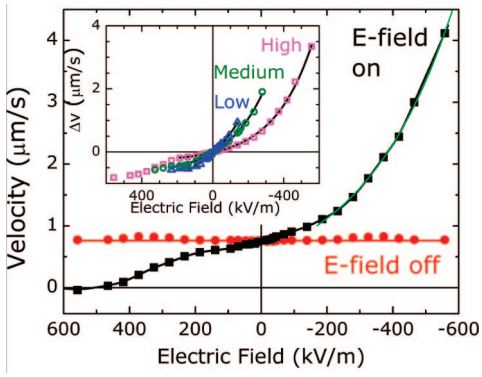

Figure 2. Microtubule velocity as a function of electric field. Black squares are the velocity of microtubules measured under different applied electric fields. Red dots denote the determined microtubules velocity under zero fields measured just after the corresponding black dot. Each point corresponds to an average of several microtubules. The green line is an exponential fit to the data at high fields. The inset presents the velocity increase, $\Delta v(\mathbf{E}) = v(\mathbf{E}) - v(0)$, for three different channels. Each curve relates to a different surface kinesin density.

comparison to the behavior at zero field, the microtubules show a strongly increased velocity for assisting forces (negative fields, +100 and +200 V), whereas the motility gets reduced for opposing forces (positive fields, −100 V). The effect of the applied electric field on the average velocity is displayed in Figure 2. This figure shows that the applied electric field can strongly increase the gliding velocity of the microtubules or decrease it to zero (black dots). At the largest assisting forces studied, the velocity has increased from about 0.8 μm/s (at zero field) to about 4.0 μm/s. For microtubules that run in the direction against the electrical force, the speed decreases with increasing field strength, and the gliding microtubules can even be stopped at a field strength of about +500 kV/m. Note that the zero-field velocity measured just after the applied fields were switched

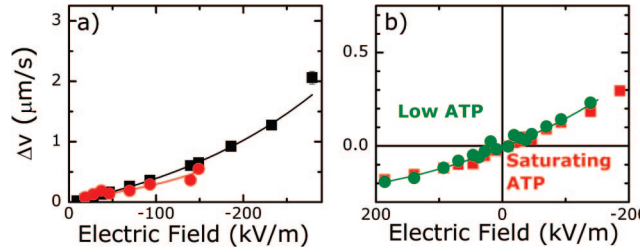

Figure 3. Velocity change $\Delta v(\mathbf{E})$ under different experimental conditions as function of electric field. (a) Velocity change parallel (black squares) and perpendicular (red dots) to the electric field. Both sets of data overlap. (b) Velocity change under saturating ATP level (red square) and low ATP (green dots) concentration. The velocity change does not depend on the ATP concentration.

off is not modified throughout the experiment (red dots). For large negative fields and thus positive assisting forces, $E < -200$ kV/m, the velocity appears to be well fitted with an exponential growth $v = v_0 e^{\mathbf{E}/\mathbf{E}_0}$, with $v_0 = 540$ nm/s and $\mathbf{E}_0 = -270$ kV/m. The velocity is defined as positive if the microtubule velocity is such that its minus end is leading (i.e., the normal propagation mode at zero field). The statistics for the microtubules under opposing forces are somewhat lower because most of these gliding microtubules change their direction of motion after some time under the applied fields due to tip bending changes the original direction of motion. The slowing down and stalling is in agreement with previous studies on single kinesins.[11] By contrast, the significantly increased speeds have, to our knowledge, not been observed before.

The inset to Figure 2 shows the similar behavior in three different channels that were independently prepared. Here, we display the velocity changes Δv, defined as the difference between the applied-field velocity and the zero-field velocity. Qualitatively, the same behavior is observed in the three channels, however, with differences in the rates of change. Although the channels are prepared in the same way, the kinesin density changes from sample to sample. We therefore attribute the different rates to different surface kinesin densities in the channels, where a higher kinesin density requires a larger force to stop the gliding microtubule.

The velocity increase was not only observed for microtubules running parallel to the field. Remarkably, for microtubules gliding perpendicular to the field, as shown in Figure 3a, we also observe a significant velocity component that is directed perpendicular to their axis and parallel to the electric field. In this figure we observe that the velocity change $\Delta v_\perp$ is similar to $\Delta v_\parallel$ of the microtubules moving parallel to the electric fields. These phenomena seem to suggest that the velocity increase is not directly an effect of a field-induced modulation of one of the steps in the kinesin ATP cycle. If that were the case, the velocity along the microtubule direction would be modified, but a velocity perpendicular to the gliding motion is not to be expected.

The magnitude of the ATP concentration seems not to have any impact on the velocity increase. We find the same Δv for saturating ATP levels (10 mM) and for a low (100 μM) ATP concentration; see Figure 3b. However, in the full absence of ATP, all motion disappears: if the ATPase enzyme

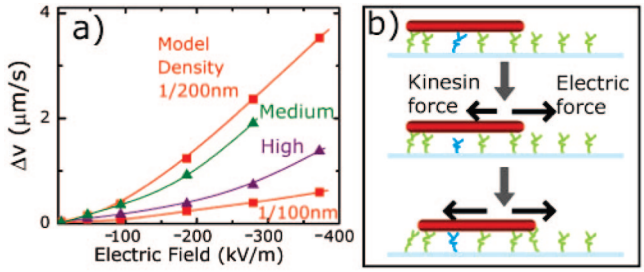

Figure 4. (a) Calculated velocity changes resulting from model simulations (red squares), compared to experimental data. The calculated velocities are obtained with the model described in the text for average kinesin densities of 1/100 and 1/200 nm. The experimental data correspond to those displayed in the inset to Figure 2. (b) Scheme describing model. If there is a detachment, the microtubule finds a new equilibrium position given by the kinesin stretching forces and the electric forces.

Apyrase is flushed in to hydrolyze all ATP in the channels, all the microtubules stop moving (velocities are reduced to <5 nm/s), even in the presence of external voltages. When, subsequently, ATP is reintroduced to the system, the microtubules regain motility. These observations show that the turnover of ATP is essential but not rate limiting in the motility increase that we observe.

How to interpret these phenomena, in particular the remarkably strong velocity increases induced by the electric fields? We suggest that the observed velocity is a combination of a normal gliding motion together with a field-modulated release and recapture process, where kinesins step, release, and rebind to microtubules, and microtubules get pushed due to the external forces while this is happening. If the latter occurs faster than the release and rebind, motion occurs and the microtubule will travel considerably faster. In order to test this proposal, we developed a simple model which describes a gliding motion with external forces applied to the microtubules due to the applied electric fields (Figure 4b). In this model, the three-dimensional problem is mapped onto a one-dimensional problem, where the positions of kinesins that can reach the microtubule are projected into a one-dimensional line. The microtubule then glides on top of a randomly distributed linear array of kinesins. The kinesin molecules are considered as springs with a spring constant of $k = 0.2$ pN/nm[12] that can bind to the microtubule if the microtubule lies within 30 nm from the anchoring point of the kinesin. Dwell times between steps are taken to be 10 ms, and the binding times t_0 are made to depend on the amount that a kinesin is stretched, $t = t_0 e^{-kd\delta_L/k_B T}$,[9] where kd is the force on the microtubule given by the stretching d of a single kinesin, δ_L is the characteristic distance associated with the load dependence (1.3 nm),[13] and t_0 is the binding time of kinesin without stretch ($t_0 = 1.25$ s). We constructed the force-dependent binding time of a single kinesin motor (i.e., its processivity in units of time) by dividing its force-dependent run length (measured by Block et al.[9]) by its force-dependent velocity (measured by Schnitzer et al.[13]). Note that in this model we have fixed the dwell time and thus do not include the ATP concentration as a parameter. We run simulations where at each iteration of the program the positions of the attachment points of each kinesin to the microtubule are determined and the force on each of them is checked. Microtubules are taken to be 5 μm long rigid rods, on which forces act due to the external applied field as well as due to the stepping kinesins. After a step of one of the kinesins, we probe if there is a detachment either due to overstretching of certain kinesins (beyond 30 nm) or as a result of a random detachment event. Upon detachment of one or more kinesins, the balance is disturbed between the external electric force and the restoring elastic forces exerted by the kinesin molecules. As a result of this imbalance, the microtubule will move in the direction of the electric force, thereby further stretching the remaining kinesins until force balance is restored (Figure 4b). Thus, after each detachment event, a new microtubule position is calculated from force balance. We neglect the time needed for reaching the new equilibrium position, which is a reasonable assumption if this time is much shorter than the time between detachment events. We estimate that the time needed for reaching the new equilibrium position is $\sim$10 μs, which is much smaller than the typical time between detachment events, thus validating the approximation.[14]

Figure 4a shows some results from the simulation model and compares these with experimental results. We observe that our simple model displays similar trends as the experimental data, that is, the velocity increases strongly and nonlinearly with the field and the magnitude of the velocity increase depends on the kinesin density. Our model suggests that the electric field modulates release rates which in effect leads to a lower processivity. A similar model for the perpendicular motion showed similar velocity increases with the applied field.

The experiments presented in this work show a way of manipulating the microtubule gliding speed by the application of uniform external electric fields. Not only stopping of the movement can be achieved but also enhancements of the speeds of up to five times their normal gliding speeds. This is an important step toward the application of these motors as shuttle cargos in microfluidic environments.

Supporting Information Available: A description of nanofabrication and a figure showing a sketch of the channel pattern on the chip and details of the channel dimensions. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) The time needed for reaching the new equilibrium position can be approximated as $t = \Delta x/(\mu_{||}\mathbf{E})$, where Δx is the distance that the microtubule moves to restore force balance, which equals the extra stretching of the kinesin molecules. $\mu_{||}$ is the parallel electrophoretic mobility of the microtubule, and $\mathbf{E}$ is the electric field. Typically, $\Delta x \sim 1-10$ nm and $\mu_{||}\mathbf{E} \sim 3 \times 10^{-8}$ m^2/V s $\times$ 50 kV/m $\sim 10^{-3}$ m/s, thus $t \sim 10$ μs.

NL801837J