Probing friction in actin-based motility

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Abstract. Actin dynamics are responsible for cell protrusion and certain intracellular movements. The transient attachment of the actin filaments to a moving surface generates a friction force that resists the movement. We probe here the dynamics of these attachments by inducing a stick-slip behavior via micromanipulation of a growing actin comet. We show that general principles of adhesion and friction can explain our observations.

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Actin polymerization and assembly can build a gel, which is able to push forward the cell membrane and propel intracellular objects by actin comet formation. Biomimetic systems of actin-based motility have been useful for analyzing the physical aspects of motility mediated by actin polymerization. The experimental actin-based propulsion system that we use here is inspired by Listeria motility in cells, and consists of a polystyrene bead coated with an activator of actin polymerization. The bead is placed in a mixture of purified proteins (the complex Arp2/3, gelsolin, ADF and profilin) that reconstitute actin-based movement. In these conditions, a comet grows from the bead surface and propels the bead in a manner that is reminiscent of Listeria motility [1]. All proteins from the mixture are now available commercially from the ‘Cytoskeleton’ company (Denver) [2]. The actin comet that grows from the Listeria or bead surface is a gel made of actin filaments branched by the protein complex Arp2/3. This gel has elastic properties that have been characterized in various situations in vitro and in vivo. When it polymerizes and assembles from the bead or Listeria surface, the gel undergoes elastic deformations and the elastic stresses are relaxed to produce movement [3]. The surface of the bead mimics well the surface of Listeria, which is rigid due to the presence of a peptidoglycane layer, and carries an activator of actin polymerization which is unable to diffuse on the surface. In both cases, the gel has to glide on a rigid surface, to which it is attached. The gel exerts therefore a friction force on the bead, since friction is a general mechanism that occurs between two solid or elastomer surfaces [4]. In a certain range of parameters, we observe a stick-slip motion that we relate to the friction mechanism that was proposed in [5].

The elastic energy produced by gel deformation and dissipated by motion leads to a propulsive force of a few nanoNewton [6]. In stationary situations, the relation between force and velocity can be determined by imposing the force. However, there are regimes of non-steady movement observed in biomimetic systems [1] as well as in cells [7]. On the actin-based system studied here, a transition is observed from a continuous to a saltatory movement where the force, the velocity, and the actin gel density oscillate at the same period. We analyze the transition from continuous to oscillating movement and show that, in the probed range of force, the movement is driven by the friction force that thus varies necessarily non-monotonously with the bead velocity. Note that oscillations in actin-based movement have also been observed in other systems such as oil droplets [8] or liposomes [9]. However, these systems are different from the solid beads studied here, since the activators of actin polymerization can move on the surface whereas they are fixed here, and retain the movement, thus producing friction. Oscillations on soft surfaces do not involve any friction and their mechanism can be explained by the existence of cycles between diffusion, attachment, convection and detachment [8].

1. Materials and methods

1.1. Micropipettes

Micropipettes (Fisher Scientific) are prepared with a pipette puller (P 2000 Sutter). Their tip is broken by insertion in a molten drop of soda lime glass using a microforge (Narishige, Japan) with a 60X objective (Olympus) to allow inner tip diameter control. Micropipettes designed for bead holding have an inner diameter of about 1.5 μm. Micropipettes used to functionalize beads are 4.5 μm in diameter. The tip of micropipettes used to hold the actin tail is narrowed, by an additional heating step, to 1.5 μm internal diameter, with a surrounding 3.5 μm discoid wall.
1.2. Glass fibers

Two different fibers are obtained from either glass rods, or glass wool microfibers. Glass rods (1 mm diameter, World Precision Instruments) are pulled to a tapered length of 1.5 cm. The tip of the rod is broken at a 1.5µm diameter using the same process as for micropipettes. The tapered rods are etched in HF (10% in water) for 9–12 min to reduce the end diameter to tens of micrometres in a reproducible way. Microfibers from glass wool (Lauscha Fiber, Germany) are dispersed in water and dried on a microscope slide. One microfiber (100–500 µm long, and about 0.3 µm diameter) is caught with a glass microneedle and inserted into the tip of a micropipette (inner tip diameter of 3 µm) filled with optical glue. The microfiber is then immobilized by polymerizing the glue.

1.3. Optical bonding

Optical glue (NOA68 Norland Products) is used to hold microfibers in micropipettes, to attach the beads to the fibers and to fix platinum wires to microneedles. The laser beam from a UV pulsed laser (337 nm, Laser Science) is expanded by a telescope and focused to a 4 µm spot by a 40X UV objective (Olympus). The glue is polymerized under UV-irradiation for 5 s.

1.4. Force probe calibration using a platinum filament as a reference spring

A 1 cm long silver coated platinum wire (Wollaston, outer diameter: 100 µm, inner platinum sheath diameter: 0.6 µm, Goodfellow) is placed on a microscope slide and held on the surface by fixing its extremities with PDMS ribbons that stick to the glass. A platinum filament is obtained by removing the silver outer sheath by a 70% nitric acid treatment for 1 h, then rinsing and drying. It is then cut into a 200 µm long filament by UV laser ablation under the microscope, and fixed perpendicularly to a microneedle by optical gluing. Its rigidity is measured with the thermal fluctuation method. The image of the wire is projected on a 2 quadrants photodetector (UDT sensors) with a 1000X magnification. Position fluctuations are recorded (with a precision of 0.2 nm) and analyzed with Labview software (National Instruments). The rigidity is obtained by fitting the fluctuation power fast Fourier transform (FFT) spectrum with a Lorentzian (in the 10 Hz to 3 kHz frequency range). The rigidity of the filaments ranges from 0.2 to 0.8 nN µm⁻¹, with a precision ranging from 3 to 20%, depending on the filament. To measure the flexible handle rigidity, the end of the platinum filament is placed against the handle at the position of the bead. While the handle is kept at a fixed position, the micropipette carrying the attached filament is translated on a 10 µm course with 0.2 µm increments using a piezoelectric displacement (LISA, Physik Instrumente Germany). At each step the fiber deflection is measured by tracking the bead position with the track object component of Metamorph software (Universal Imaging). The rigidity $k'$ of the handle is derived from the slope $p$ of the deflection versus displacement plot and the rigidity $k$ of the platinum filament using $k' = k(1 - p)/p$.

1.5. Experimental set-up

Once attached on a microfiber, a 2 µm diameter polystyrene bead (Polysciences) is coated by purified N-WASp (0.75 µM solution in buffer X) for 10 min, then rinsed in a 1% BSA solution in buffer X. Experiments are performed in a PDMS/glass chamber, built as follows. A 1 mm thick PDMS rectangle (2 × 1.5 cm) is deposited on a microscope slide and a 1 mm large
L-shape channel is cut. The chamber is filled with 25 µl of standard motility medium made from pure proteins at the following concentrations: 0.1 µM Arp2/3, 0.1 µM gelsolin, 4.2 µM ADF, 2.6 µM profilin, and 7.4 µM actin. The optical visualization is made using a 60X/air phase contrast objective (Olympus) mounted on an inverted microscope (IX51 Olympus) and images are taken by a cooled CCD device (Cool Snap, Princeton Instruments).

1.6. Experiment automation

For any kind of experiment, the force is measured by tracking the bead position every ~0.3 s on small images (size 10 × 5 µm² approximately) centered on the bead using the ‘track object component’ of Metamorph software (Universal Imaging). The zero force is determined by locating the bead position on the small image before comet handling. Every 30 steps (9 s approximately), a large image (50 × 40 µm) is taken to visualize the handle and the entire comet.

For fast pulling experiments, the micropipette is displaced by an amount proportional to the imposed velocity and the time interval between two tracks.

For force–velocity experiments, a feedback loop is used to apply a constant force on the system (equivalent to a constant deflection). For each step, the proportional feedback loop (custom program) calculates the displacement to apply to the micropipette to correct the deflection that is imposed within a 30 nm error. The maximal correction speed is 18 µm s⁻¹.

1.7. Comet dimensions measurements

The length of the actin tail equals the distance between the bead and the micropipette and is measured manually on the image with a precision of 0.1 µm.

2. Results

We follow the dynamic behavior of actin-based movement by holding the bead–comet system between a flexible microneedle and a micropipette. The bead is firmly attached to the microneedle and the comet is held in the micropipette by a slight aspiration. The microneedle allows a force $F_{\text{ext}}$ to be applied, and the micropipette is displaced at an imposed speed $V_{\text{pull}}$ (figure 1(A)) higher than the natural growth velocity of the comet [6].

When $V_{\text{pull}}$ ranges between 4.5 and 5 µm min⁻¹ (larger than the natural velocity of 2 µm min⁻¹ in the absence of external force), the force $F_{\text{ext}}$ transiently increases up to a maximum, then reaches a stationary value for which the comet growth velocity equals the pulling speed. The comet density is homogeneous along its elongation axis and decreases when the growth velocity increases (see figure 1(B)). For pulling speeds larger than 5.5 µm min⁻¹, both force and velocity display a clear oscillating behavior with a period of about 1 min (figure 1(B)). The force as a function of time has a saw-tooth behavior, with a slow increase to a maximum followed by fast decrease associated with a peak in the velocity. The comet density is higher at low velocities (increasing force), than at large velocities (decreasing force) (see movie S1, available from stacks.iop.org/njp/9/431/mmedia, the gel generated from the bead surface is denser, respectively, less dense, when the microneedle moves to the right, respectively, to the left). The oscillation amplitude increases with the pulling speed.
Figure 1. (A) Scheme of the experiment. The flexible microneedle is represented as a spring. The comet is pulled with a constant speed $V_{\text{pull}}$. The applied force $F_{\text{ext}}$ is measured by the deflection $x$. The comet grows at a velocity $V$. (B) Force (blue) and growing velocity (red) are plotted over time for different pulling speeds. Each black line corresponds to 1 nN for the force and to the pulling speed indicated on the right. The small oscillations in each curve are due to the measurement process. The corresponding image of the comet at the end of the pulling experiment is shown on the right of each curve (scale bar 10 $\mu$m). (C) Force–velocity curve corresponding to the pulling at $V_{\text{pull}} = 5.5 \, \mu$m min$^{-1}$. 

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3. Discussion

In addition to the external force $F_{\text{ext}}$ applied on the comet by the microneedle, two other forces are at work \cite{5}: the elastic force $F_{\text{el}}$ generated by the actin gel growth on the spherical bead and the opposing friction force $F_f$ between the comet and the bead. In a stationary state, using the model developed in \cite{5}, the elastic force reads $F_{\text{el}} \cong E \frac{e^3}{R}$ \footnote{Note that in principle we should use $F_{\text{el}} = E (e^3/R)(1 + e/2R)$. We checked that this does not lead to any significant change in the data analysis.} where $E$ is the elastic gel modulus, $e$ the gel thickness on the side of the bead and $R$ the bead radius. The expression of the gel thickness can be expressed as

$$ e(V) = e^* \left[ 1 - \exp \left( - \frac{V_p R}{V e^*} \right) \right], $$

where $v_p$ is the polymerization velocity, $V$ the bead velocity, and $e^*$ is obtained in spherical geometry in the absence of symmetry breaking \cite{6,11}. At sufficiently high velocities, we obtain that $F_{\text{el}} \approx E R^2 (v_p / V)^3$. Using an actin polymerization velocity of 1.6 $\mu$m min$^{-1}$ \footnote{New Journal of Physics 9 (2007) 431 (http://www.njp.org/)}, an elastic modulus of $10^3$ Pa, and a bead velocity of 4.5 $\mu$m min$^{-1}$ that corresponds to the smallest value in our experiments, we obtain that it is less than 30 pN; this value is small compared with $F_{\text{ext}}$ of the order or less than a few nanoNewtons. The elastic force is therefore negligible and the oscillatory bead motion results from a balance between the external force $F_{\text{ext}}$ and the friction force $F_f$. In order to account for the oscillations, the friction force must then be a non-monotonous function of the velocity $V$ and have a decreasing unstable branch. The experimental plot of as a function of $V$ (figure 1(C)) indeed displays cycling between two branches. Positive friction due to an attachment/detachment of linking proteins has been first discussed by Tawada and Sekimoto \cite{13}. The extension to the negative friction regime has been derived in \cite{5} and discussed more recently in \cite{14} for the relative motion of two solid surfaces separated by an adhesive surfactant. This analysis leads to a stick-slip phenomenon, similar to the one observed here. In this description, smooth movement occurs when an average number of filaments remain attached whereas stick-slip motion occurs when a cooperative breaking happens. Note that this type of friction is different from the ‘classical’ view of friction where stick-slip disappears at high velocity. Here, we explore the low velocity branch first where continuous motion occurs, and show evidence of a stick-slip phenomenon at intermediate velocity. There might be a regime of continuous motion at higher velocity, but it is inaccessible experimentally. Alternatively, the non-monotonic friction could be due to a strong decrease of the gel density with velocity that would lead to a decrease of the friction force with velocity and explain the gel density oscillations observed experimentally.

Our work paves the way for further studies of the dynamics of proteins that connect actin filaments to the surface and promote directed actin polymerization, since molecular details can be inferred from mesoscopic behaviors and vice versa.

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