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Magnetic manipulation of actin orientation, polymerization, and gliding on myosin using superparamagnetic iron oxide particles

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Abstract

The actin cytoskeleton controls cell shape, motility, as well as intracellular molecular trafficking. The ability to remotely manipulate actin is therefore highly desirable as a tool to probe and manipulate biological processes at the molecular level. We demonstrate actin manipulation by labeling actin filaments with superparamagnetic iron oxide particles (IOPs) and applying a uniform magnetic field to affect actin orientation, polymerization and gliding on myosin. We show for the first time magnetic manipulation of magnetizable actin filaments at the molecular level while gliding on a bed of myosin molecules and during polymerization. A model for the magnetic alignment and guiding mechanism is proposed based on the torque from the induced molecular anisotropy due to interactions between neighboring IOPs distributed along magnetically labeled actin molecules.

1. Introduction

Actin is critical for many cellular attributes: morphology, motility, mitosis, and molecular transport [4]. Actin polymerization, depolymerization and organizational arrangements (branching, bundling) are highly regulated; dysfunctions in such regulation are involved in a variety of pathologies [2] including cancer [11], cardiovascular disorders [13, 9] and immunodeficiency [5]. Manipulating the orientation of the polymerized actin filaments and/or polymerization itself may facilitate altered cellular behaviors. For example, it was shown recently that the local activation of a myosin-dependent signaling pathway can control the directionality of cell movements in a highly specific manner [15]. Moreover, controlling how cytoskeletal elements behave may be useful in engineering applications that require molecular patterning [1]. The choice of magnetic over electric fields or light for actin manipulation is based on the long term goal of controlling cell function in tissues where the penetration of electric fields (due to shielding) or light (due to absorption) is limited. Actin filaments decorated with nano-sized superparamagnetic iron oxide particles (IOPs) were first synthesized and functionally tested as a viable nanomaterial by Patolsky *et al*, making the possibility of precise patterning and manipulation of magnetizable actin filaments through

magnetic fields probable [12]. There have been reports on manipulating the orientation of gliding microtubules (polymerized actin molecules), either by external magnetic fields [3] or electric fields [8]. Magnetic manipulation of actin filaments at single molecule scale, however, has yet to be systematically established. Here it is also demonstrated that magnetizable actin filaments could be aligned or directed when gliding and polymerizing.

2. Experimental details

2.1. External magnetic field

A pair of neodymium magnets (BYOX08BR, K&J magnetics) was mounted on an aluminum plate. The permanent magnets face each other with opposite poles and are 12 cm apart. According to the manufacturer, the magnet used here has a surface field of 3900 Gauss and residual induction of 13 200 Gauss. The magnetometer reading showed that the field strength is ~ 0.4 T. The configuration is shown in figure 2(a).

2.2. Incorporation of IOP into F-actin

The labeled and unlabeled g-actin monomers used in this study were a generous gift from Dr Ikuko Fujiwara (National Institutes of Health). In order to produce functional actin filaments that are magnetizable, 5% biotinylated g-actin and 5% rhodamine-labeled g-actin were mixed with unlabeled g-actin monomers (90%), making a final total actin concentration of 1 μ M, for polymerization. The polymerization was performed for an hour in buffer containing 0.05 M KCl solution, 1 mM $MgCl_2$ and 1 mM EGTA. Subsequently, 10 nm iron oxide particles conjugated with streptavidin (SHS-10-01, Ocean NanoTech) were added to the mixture at 1:1 ratio to the biotinylated g-actin.

2.3. Magnetic manipulation chambers for microscopic imaging

Iron oxide particle-incorporated f-actin filament (IOFA), or the mixture of g-actin and IOPs in the case of on-stage polymerization experiments, was injected gently through a thin chamber made of two 0.1 mm thick coverglasses coated with myosin V, a generous gift from Dr James Seller (National Institutes of Health). The preparation of myosin coating is described in the section on gliding assay. Images were acquired before and after the sample chamber was placed in the presence of magnetic fields. In the alignment experiments, movie recording was performed in the presence of 0.5% methylcellulose (M 0262, Sigma-Aldrich).

2.4. Gliding assay

Gliding chambers were pre-blocked with 0.01 mg ml⁻¹ BSA before myosin V was added at the concentration of 0.5 mg ml⁻¹. The myosin was incubated in the chamber for 5 min. 20 μ l of IOFA (8 nM) was flowed through the chamber subsequently, followed by washing with motility buffer (10 mM imidazole, pH 7.2, 75 mM KCl, 1 mM EGTA, 2.5 mM $MgCl_2$, 10 mM Mg-ATP, 8 mM DTT, 0.2 mg ml⁻¹ glucose oxidase, and 0.1 mg ml⁻¹ catalase). IOFA motility was observed after the addition of ATP (10 μ M) using a fluorescence microscopy. The acquisition frame rate was 1 Hz.

2.5. Microscopy

An Olympus IX71 microscope was used for the optical imaging in this study. For electron microscopy, IOFA samples were dropped onto an EM grid and air dried subsequently to be imaged by a JEOL 1200 EXII transmission electron microscope.

2.6. On-stage actin polymerization

G-actin monomers labeled with IOPs, biotin and unlabeled monomers were mixed at 1:1:18 ratio with a total volume of 20 μ l in a chamber made of 0.1 mm thick coverglasses with a 100 μ m thick spacer in between. The chamber was placed between the magnets and time-lapse images were taken to record the growth of F-actin. The control experiment was performed without the presence of an external magnetic field. The acquisition rate was 1 frame min⁻¹.

2.7. Simulation and image analysis of actin orientation

A Matlab code was created to simulate the random arrangement or alignment of F-actin. The Fourier transformation was performed using the ImageJ program provided by NIH.

3. Results and discussion

10 nm diameter IOPs were attached to F-actin during polymerization. The incorporation of IOPs into actin filaments was confirmed by electron microscopy (figures 1(a) and (b)). The IOPs are composed of an iron oxide core and a layer of dextran coating, onto which streptavidin is grafted to functionalize the particles. The actin filaments were also stained with anti-dextran antibody that targets the dextran coating on the IOPs (figure 1(c)). The IOP-incorporated F-actin (IOFA) was then subjected to a series of magnetic manipulation experiments as described below.

IOFA was gently injected into a microfluidic chamber coated with myosin V and subsequently placed in a uniform external magnetic field. The degree of IOFA alignment with the magnetic field was imaged first with the field off then with the field on using fluorescence microscopy. It was noted that most actin filaments were aligned in parallel to the magnetic field (figures 2(b) and (c)), whereas the control actin filaments without IOPs did not show alignment (data not shown).

Some actin filaments were not straight and thus it was difficult to directly measure the angle relative to the magnetic field. Therefore to assess the degree of alignment, the arrangement of actin filaments in both random and perfectly aligned states (images shown in figures 3(a) and (b)) was simulated as the ideal reference state. 2D Fourier transform was performed on the simulated images (figures 3(c) and (d)). Similarly the experimental images before and after the magnetic field application were also processed for comparison. The Fourier transform pattern before magnetic manipulation resembles the pattern of a simulated random distribution. In contrast, the pattern after magnetic manipulation has a distinctive, longer axis similar to the one resulting from the simulated aligned lines. Quantitatively, the ratio between the average intensity in the region close to the y axis (the small yellow box indicated in figure 2(d)) and that of the bigger area (the big red box indicated in figure 2(d))

was seven-fold higher in the aligned F-actin compared to the control, while it was ten-fold higher in the case of complete alignment depicted by simulation, indicating that there was significant alignment in the presence of a magnetic field. It is noted that not every IOFA was aligned in parallel to the magnetic field. This might be due to the limitation in our protocol to synthesize IOFA, where conjugated IOPs are not uniformly distributed (figure 1). Therefore not every actin filament was attached with enough IOPs to generate the necessary magnetic torque required for the alignment.

To elucidate the mechanism behind the alignment in the presence of a uniform magnetic field, methylcellulose buffer, which has 100-fold higher viscosity (10^{-1} Pa s) compared to water, was employed to dampen the rapid motion of the actin filaments so that the aligning process could be recorded in a detailed manner. It was observed that IOFAs can align to the field lines through a magnetic torque-driven rotation where a limited fraction of the actin filaments stayed attached to the substrate, serving as pivot, while the free part of the filament rotated until it was aligned to the magnetic field (figure 4(a), movie 1 (available at stacks.iop.org/0957-4484/22/i=6/a=065101/mmedia)). From electron microscopy, it was shown that the IOP–actin conjugation resulted in uneven spacing between the iron oxide particles (figures 1(a) and (b)), that is, the distance between neighboring particles is not with regular intervals but rather exhibits patterns of aggregation, usually with more than five particles arranged closely with the neighboring particles. The characteristic clustering in theory enables the groups of iron oxide particles to behave like semi-rigid rods in the presence of an external magnetic field. The torque responsible for the rotation would then result from the self-induced magnetic anisotropy of the short chains of IOPs distributed along the actin molecule. Sparsely distributed magnetic particles will form short rod-like chains in the presence of an external magnetic field. This leads to optical birefringence in an external applied field due to the induced magnetic anisotropy of the short chains causing alignment with the applied field [6, 7, 10]. We believe that a similar phenomenon is occurring for magnetic chains attached to the actin molecules. In principle the maximum torque leading to alignment would be

$$\Gamma_{\max} = m_p \frac{\langle m_{pc} \rangle}{m_p} \langle n \rangle B, \quad (1)$$

Where m_p represents the magnetic moment of a particle, $\langle m_{pc} \rangle / m_p$ represents the mean reduction of the magnetic moment of the particle in the chain [14], n represents the mean number of particles in the chain. Specifically, in our case, we estimate a $\Gamma_{\max}$ of 2.3×10^{-19} N m given $m_p = 2.3 \times 10^{-19}$ A m², $\langle m_{pc} \rangle / m_p > 0.5$ [6], $\langle n \rangle = 5$, and $B = 0.4$ T. The magnetic torque should be high enough to overcome thermal fluctuations. The highest energy configuration is a rotation of the chain through 90° ($\pi/2$ radians) relative to the applied field or $E_\Gamma = \pi/2 \times \Gamma_{\max} = 3.7 \times 10^{-19}$ J compared to $ET = K_B T = 4.1 \times 10^{-21}$ J for $T = 300$ K given $K_B \equiv 1.38 \times 10^{-23}$ J K⁻¹. Based on thermal energy estimates we conclude that at room temperature alignment of five particle long chains with the applied field will be thermally favorable, suggesting that any chain of five particles would be

oriented to the field to within 1°. In addition, free sections of the actin molecules will experience Brownian forces as well [16]:

$$F_{\text{thermal}} = \frac{K_B T}{V^{\frac{1}{3}}}, \quad (2)$$

where v represents the volume of the object. The chain length l_{chain} for a five particle (10 nm diameter) chain would be 50 nm and so it is estimated that $v = \pi \times (5 \text{ nm})^2 \times 50 \text{ nm} = 3.9 \times 10^{-24} \text{ m}^3$ and further concluded that at room temperature $F_{\text{thermal}} = 2.6 \times 10^{-13} \text{ N}$. A Brownian torque at a given instant could be possibly as large as

$\Gamma_{\text{thermal}} \frac{1}{2} F_{\text{thermal}} l_{\text{chain}} = 1.3 \times 10^{-20} \text{ N m}$. This is also less than the magnetic field alignment torque estimate of $\Gamma_{\text{max}} = 2.3 \times 10^{-19} \text{ N m}$. Based on torque ratios ($\Gamma_{\text{max}} \sin \theta = \Gamma_{\text{thermal}}$) we conclude that any chain of five particles would be oriented to the field to within 5°.

It is also notable that when a protein has an alpha-helix structure, the total susceptibility is determined by the vector addition of the individual magnetic susceptibilities along the principal axes, rendering a much higher value than expected [10]. It had been observed that unlabeled microtubules might align by an external magnetic field to a degree [14] without the need for IOPs, indicating the possibility of induced magnetic moment and anisotropy in proteins themselves. Although we did not observe alignment of unlabeled actin filaments it is plausible that the magnetic torques of the two origins join (magnetized IOPs and from the magnetized protein chains), contributing to the torque that aligned the IOFAs to the external magnetic field. Another possibility is that in addition to the uniform applied magnetic field there is a field gradient that attracted the IOFAs to move directionally. This is unlikely since the magnetic force generated by the gradient, which was negligible in our configuration near the center of the pair of magnets, would be too small to overcome thermal fluctuations.

Myosin/ATP-facilitated gliding of IOFA was also examined. The myosin V molecules were bound to the glass surface of the microfluidic chamber. Biased ATP-dependent gliding along the magnetic field lines was observed (figure 4(b) and movie 2, available at stacks.iop.org/0957-4484/22/i=6/a=065101/mmedia) whereas the gliding was randomized without the applied magnetic field (figure 4(c) and movie 3, available at stacks.iop.org/0957-4484/22/i=6/a=065101/mmedia). The biased gliding observed is probably due to torque-driven rectification of the front segment of an IOFA, which was free momentarily before being grasped by a nearby myosin (for the model see figure 4 (d)). The mechanism of rectification probably involves the torque-driven alignment on the free IOFA segment at the leading end with a pivoting point where a myosin molecule is in contact with the actin filament. After the free IOFA segment in the leading front is aligned to the field, the myosin molecule at the proximity binds to the segment to perform a power stroke pushing the filament forward. The stroke cycle will repeat again when the myosin releases the leading IOFA segment again to be realigned with the field once more.

Finally the polymerization of actin filaments was recorded with or without the presence of an external magnetic field. The growth of F-actin was imaged in a chamber containing the g-actin monomer mixture (biotinylated, rhodamine-labeled and unlabeled g-actin) and

streptavidin-coated IOPs for IOFA formation in time-lapse acquisition mode. It was observed that the external magnetic field aligned the orientation of the polymerization to the field (figure 5 and movie 4, available at stacks.iop.org/0957-4484/22/i=6/a=065101/mmedia) whereas no such bias was detected in the control (movie 5, available at stacks.iop.org/0957-4484/22/i=6/a=065101/mmedia).

4. Conclusion

Our study showed that with 5% biotinylated g-actin monomers conjugated to streptavidin-coated, 10 nm IOPs, the orientation, gliding, and polymerization of F-actin could be controlled using a uniform magnetic field. The capacity to manipulate these three types of actin behavior should provide some intriguing possibilities for controlling actin in future applications. In addition to manipulating cell physiologies, one potential *in vitro* application is to use actin as a nano-patterning vehicle by constructing an actin-based track system where a specific pattern is built using magnetic torque to program the routes of motor proteins and therefore control the delivery of substances carried by the motor proteins. The choice of actin over microtubules for nano-patterning has merits since actin is smaller in size and requires less strict conditions to polymerize and stay polymerized. Currently we are investigating spatially addressable magnetic field patterns in a more confined manner with ways to locally switch magnetic fields on and off as well as ways to locally change field direction at the nanoscale.

Trade names have been used in the text for clarity: NIST does not endorse any commercial products or methods described in the paper.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

The incorporation of IOPs into actin filaments was examined by transmission electron microscopy. The 10 nm iron oxide particles are shown as the dark circles bound to the F-actin which has lighter, filamentous structure shown in the micrographs of $30\,000\times$ (a) and $50\,000\times$ (b) magnification. Selective examples of IOFA are marked by white closed curves. The IOP distribution at each actin filament varies in both density and spacing. The IOP conjugation was also verified by the immunostaining of the F-actin targeting the dextran coating of IOPs (c).

Figure 2.

IOFA is aligned with the external magnetic field. (a) The chamber containing actin is placed on top of a $100\times$ objective of an inverted microscope. The permanent magnets face each other with opposite poles, forming a uniform field in the center where the samples were placed. (b) Before the sample was placed in the parallel magnets, IOFA was randomly arranged (left); IOFA became aligned with the magnetic field after being placed between the two parallel magnets (right). (c) IOFA was sparsely spaced and imaged with lower magnification ($20\times$). In the presence of magnetic field (right) $\sim 85\%$ of the filaments became aligned. (d) The 2D Fourier transform was performed on the images in (b); the upright spindle pattern is prominent in the 2D Fourier transform of the horizontally aligned F-actin image. Also note that the brightness along the x- and y-axes of the 2D spatial Fourier transform represents the narrow width of the F-actin in both dimensions of the spatial domain. The visual difference was assessed by calculating the brightness ratio between the areas inside the small yellow and the big red boxes in aligned actin (right) versus that in the randomly distributed actin (left). The average value in the aligned samples, from three independent experiments, is seven-fold higher (with a standard deviation of 0.9) than the control, indicating a high level of alignment of F-actin (see figure 3 for comparison).

Figure 3.

Simulation of randomly arranged and aligned F-actin. Matlab codes were created to simulate the arrangement of F-actin. Parameters such as the number and the average length of the actin filaments in the real sample were applied to generate the simulated images at the condition of random distribution (a) or complete alignment (b). A 2D Fourier transform was performed on the simulated images ((c), (d)). The visual difference was assessed by calculating the brightness ratio between the areas inside the small (yellow) and the big (red) boxes in aligned actin (d) versus the ratio between the corresponding regions in randomly distributed actin (c). The value of the former is ten-fold higher than the latter.

Figure 4.

(a) IOFA was aligned to the external magnetic field by rotation where one part of IOFA served as pivot while the rest swung until alignment to the field (black arrow) was achieved (also see movie 1 available at stacks.iop.org/0957-4484/22/i=6/a=065101/mmedia). (b) Biased F-actin gliding was observed in the presence of the external magnetic field. F-actin glided unidirectionally along the x-axis (transient directions are indicated by white arrows) when placed between two magnets (also see movie 2 available at stacks.iop.org/0957-4484/22/i=6/a=065101/mmedia). (c) IOFA glided randomly (transient directions are

indicated by white arrows) without external magnetic field (also see movie 3 available at stacks.iop.org/0957-4484/22/i=6/a=065101/mmedia). The images in (b) and (c) were extracted from time-lapse movies recorded at 5 frames s⁻¹ rate at time points 0, 35, and 69 s, as indicated. The hypothesized mechanism of biased gliding of IOFA in the presence of an external magnetic field is shown in (d), where the free leading segment of IOFA is first aligned to the magnetic field **B** (bold arrow) and subsequently grabbed by the myosin in the proximity. The following stroke of the myosin pushes the whole filament forward in line with the magnetic field. The leading segment, now free again, will bind to the next myosin. The aligning, myosin-binding, forward-stroke cycle is repeated, resulting in a unidirectional movement parallel to the magnetic field. The gliding experiments with or without external magnetic field were repeated ten times each with consistent results.

Figure 5.

The polymerization of IOFA in the presence of a magnetic field is biased. From left to right, time points at 0, 14, 20 and 27 min after the sample was placed in the magnetic field. The newly formed actin filaments (indicated by dashed arrows) were aligned to the magnetic field (white arrow). The time series shown is representative of three independent polymerization experiments in the presence of the magnetic field (also see movie 5 available at stacks.iop.org/0957-4484/22/i=6/a=065101/mmedia). Twelve filaments out of 15 in total were aligned, whereas polymerization without magnetic fields showed random orientation (see supplementary movie 5 available at stacks.iop.org/0957-4484/22/i=6/a=065101/mmedia).