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Alignment of actin filament streams driven by myosin motors in crowded environments

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ABSTRACT

Background: Cellular dynamics depend on cytoskeletal filaments and motor proteins. Collective movements of filaments driven by motor proteins are observed in the presence of dense filaments in in vitro systems. As multiple macromolecules exist within cells and the physiological ionic conditions affect their interactions, crowding might contribute to ordered cytoskeletal architecture because of collective behavior.

Methods: Using an in vitro reconstituted system, we observed the emergence of stripe patterns resulting from collective actin filament streaming driven by myosin motors in the presence of the crowding agent, methylcellulose (MC).

Results: Although at high KCl concentrations (150 mM), actin filaments tended to dissociate from a myosin-coated surface, 1% MC prevented this dissociation and enabled filament movement on myosin molecules. At concentrations of actin filaments above 0.2 mg/mL, the moving filaments accumulated and progressively formed long, dense bands. The bands were spaced at about 10-μm intervals. Increasing the KCl concentration up to 300 mM resulted in narrowing of the spacing between the aligned bands. On the other hand, low KCl concentrations ($\leq$ 25 mM) induced broad streams, where actin filaments exhibited bidirectional movement.

Conclusions: These results suggest that crowded environments can promote spatial patterning of the actin cytoskeleton, depending on the intensity of the myosin driving force and filament velocity, both modulated by the ionic strength.

General significance: The mutual contribution of packing and driving forces provides insight into cytoskeleton organization in living cells, in which various macromolecules mingle.

1. Introduction

Oriented cytoskeletal structures such as stress fibers and parallel bundles are found in crawling cells [1,2]. While these structures change dynamically, a certain order is preserved to enable the directional movement. Cytoskeletal filaments in the filopodium are closely packed with cross-linker proteins to strengthen the leading edge, while the elongation and collapse of filaments in the lamellipodium occur consecutively to allow progression [3–7]. Growth cones are autonomous driving architectures whose movement does not depend on the signaling from the nucleus, and the fusion and re-orientation or re-modeling of the cytoskeleton can spontaneously occur upon contact of two growth cones [8–10]. These dynamically ordered structures illustrate how cooperative phenomena arise from stochastic events such as one-on-one interactions between proteins.

In vitro, the driving of concentrated cytoskeletal filaments such as actin filaments through ATP hydrolysis by myosin motor proteins results in a collective motion of these filaments; an ordered pattern spontaneously arises during steady movement with density fluctuation [11,12]. In addition, proteins associated with actin filaments can induce bundling of these filaments by filament cross-linking with the potential to develop a more ordered or regulated structure [13,14]. Similarly, concentrated microtubules that interact with kinesin or Ncd motors form aster-like structures [15,16]; the addition of a cross-linker and polyethylene glycol (PEG) also leads to the formation of distinct active networks and nematic streaming [17–20]. However, autonomous motion, in some cases, including collectivity, is not limited to biomolecules; physical conditions such as the Marangoni effect and viscoelastic properties of the medium result in the formation of ordered structures [21–25].

As cytoskeletal and other proteins are present at high concentrations in a cell, proteins function in crowded environments, which may enhance enzyme activity and the affinity between proteins because of a depletion force [26–28]. Furthermore, crowded environments tend to pack proteins into semi-crystals and assemble cytoskeletal filaments into bundles [29–31]. In particular, methylcellulose (MC), which can

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act as a crowder, is known to suppress the Brownian motion of actin filaments; it has been utilized in a motility assay with low myosin density, when the probability of actin-myosin attachment was reduced [32]. Similarly, MC can be utilized under conditions similar to cytoplasmic ionic strength because high ionic strength tends to weaken the interaction between actin filaments and myosin heads [33,34]. In general, ionic strength is an important factor for protein and cell functions because their electrostatic interactions are considerably affected by the ionic strength. Nevertheless, how such crowded conditions and ionic strength can contribute to the creation of ordered structures or collectivity in autonomously propelling matter remains unclear.

Here, we show that concentrated actin filaments driven by heavy meromyosin (HMM) form long streams with a regular stripe pattern in the presence of MC in relation with the effect of MC on a collective movement in microtubule-kinesin systems [35]. In addition, our results suggest that the formation of streams and the regular patterns depend on KCl concentration. The process might involve the balance between driving and depletion forces as well as the tension between different fluid layers.

2. Materials and methods

2.1. Proteins and reagents

Yamagata University Institutional Animal Care and Use Committee approved all procedures and protocols used in animal experiments. Actin and myosin were prepared from the skeletal muscles of the leg and back of a rabbit (one animal, JW/CSK, Japan SLC, Inc., Shizuoka, Japan) and HMM was prepared by chymotryptic digestion according to standard procedures [36]. Actin filaments obtained after actin monomer polymerization were labeled with tetramethylrhodamine-phalloidin (Sigma-Aldrich, St. Louis, MO, USA) [37]. The two types of MC [15 cP and 1500 cP viscosity at 2% (w/v), 20 °C] were from Sigma-Aldrich and were used without further purification. The former had an average molecular weight of 14,000 Da. The latter was used in most experiments and had a molecular weight of 63,000 Da, based on Sigma-Aldrich datasheet. MC concentration is given as % w/v throughout the text. 2,3-Butanedione 2-monoxime was of special grade from Nacalai Tesque (Kyoto, Japan).

2.2. Observation of actin filaments on HMM-coated glass slides

The experiment was performed as a conventional motility assay [36], with some modifications. A thin glass slide (24 × 50 mm^2, C024501, no. 1; Matsunami Glass Industries, Osaka, Japan) treated with 0.2% collodion solution and covered by a coverslip (18 × 18 mm^2, C218181, no. 1; Matsunami Glass Industries) constituted a flow cell with a 0.1-mm gap, which was created by using two spacers from a double-sided adhesive tape. The standard solution referred to in the text contained 25 mM KCl, 25 mM imidazole-HCl (pH 7.4), 4 mM $MgCl_2$, and 0.5% (v/v) 2-mercaptoethanol. Each perfusion was performed by using 30 μL of the specified solution. HMM solution (0.1 mg/mL HMM in the standard solution) was used to infuse the flow cell. After 60 s, unbound HMM was removed and the liquid was exchanged for a standard solution supplemented with bovine serum albumin (3 mg/mL). Subsequently, F-actin solution (0.2 mg/mL unlabeled F-actin in the standard solution containing 5 mM ATP) was used to infuse the flow cell; then, fluorescently labeled actin filaments were introduced to a final concentration of 1 μg/mL. Finally, the solution in the cell was exchanged for an ATP-containing solution [varied concentrations of KCl, 25 mM imidazole-HCl (pH 7.4), 4 mM $MgCl_2$, 1 mM ATP, 0.5% 2-mercaptoethanol, 3 mg/mL glucose, 0.02 mg/mL catalase, 0.1 mg/mL glucose oxidase, and 1% MC]. Unattached actin filaments between the coverslip and the glass slide were washed out by a double perfusion with the ATP-containing solution. Fresh solution was added into the flow cell from the right (inlet); the internal solution was removed with a filter paper from the left (outlet). Owing to shear stress, the complete perfusion of the flow cell with the highly viscous solution took ca. 2 min (flow rate of ca. 200 μm/s). Next, the flow cell was set on a stage of an inverted epifluorescence microscope (Diaphoto-TMD, TMD-EF2, objective DIC 100 ×, oil; Nikon, Tokyo, Japan). Fluorescently labeled actin filaments were visualized at 25 °C with an EM-CCD camera (DE-500, Hitachi Kokusai, Ibaraki, Japan) via a relay lens (CF PL 2.5 ×; Nikon). Sequential images were acquired at intervals of 1/15 s by a computer (Power Mac G3; Apple Co., Cupertino, CA), using a video grabber board (LG-3; Scion Co., Frederick, MD, USA) and NIH image software (National Institutes of Health, Bethesda, MD, USA). The distance between neighboring pixels corresponded to 91 nm. Velocity vectors of driven actin filaments were determined by measuring the position of the pointed ends of filaments on a xy-plane with subpixel accuracy every 0.2 s. Usually, the velocity of 30–100 filaments per screen was measured during 20 s. Sliding movement was defined as a progressive displacement without drifting and backtracking, and was assessed visually. Filaments that fluctuated backwards and forwards were excluded from the analysis during velocity determinations.

3. Results

3.1. Occurrence of ordered structures in the presence of MC and densely-arranged actin filaments

The motility of actin filaments on HMM-coated surfaces, supplied with energy from ATP hydrolysis, is affected by the ionic strength. Furthermore, the attachment of actin filaments to HMM during movement becomes weaker as the ionic strength increases with changing KCl concentration [38]. Similarly, the present study revealed that, at high KCl concentration (150 mM) and in the absence of MC, most actin filaments were dissociated from the HMM-coated surface. On the other hand, even at 150 mM KCl, MC at a concentration above 0.75% prevented the dissociation and enabled the movement of actin filaments on HMM regardless of the concentration of actin filaments (Fig. 1A). When a higher concentration of actin filaments (0.2 mg/mL) was used, these filaments autonomously formed bands (narrow streams) (described in detail in Section 3.2) in the presence of 1% MC (Fig. 1B); actin bands began to form within 5 min and persisted for 30 min. The bands tended to align in parallel to the direction of the perfusion of the flow cell with the ATP solution containing MC (see Materials and methods section). They were spaced at about 10-μm intervals. Higher concentrations of actin filaments led to more condensed bands, whereas the concentrations below 0.1 mg/mL did not promote band formation. A calibration experiment confirmed a linear relationship between actin concentration (≤ 0.2 mg/mL) and the area occupied by actin filaments bound to an HMM-coated surface (Fig. 1C). Actin at 0.2 mg/mL resulted in 13% occupancy of the HMM-coated surface area in the pre-initiation stage before perfusion of ATP solution. In addition, a similar occupancy in the absence and presence of ATP was observed with 0.1 mg/mL actin. Fig. 2 illustrates the collective streaming behavior of actin filaments during movement.

3.2. Phase transition of actin filament patterns depends on KCl concentration

Next, we focused on the KCl-dependence of the formation of actin filament patterns under set MC (1%) and actin filament (0.2 mg/mL) concentrations. We subjectively classified the patterns into four phases, although their boundaries were not clearly defined. In the presence of 0–25 mM KCl, actin filaments moved along broad streams that spontaneously occurred on the HMM-coated surface (Fig. 3A, phase 1). At intermediate KCl concentrations (50–100 mM), some streams occasionally collapsed, while others partially converged (Fig. 3A, phase 2). The streams meandered without direction and alignment. At higher KCl