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Short communication

Actomyosin II interaction modulates cell cortex stability

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Abstract

Myosin II controls the viscoelastic behavior of actin filaments, interacting with actin in an energy-dependent manner. Replacing adenosine triphosphate with adenosine diphosphate changes actomyosin sliding to cross-linking. Rheological measurements show a 3–4-fold increase of the elastic portion G' in actin filaments when myosin II is present at a molar ratio r_{MA}=1:200. This observation is supported by the demonstration of inactive myosin heads along actin filaments using atomic force microscopy.

Keywords: Actin filaments; Myosin II; Rheology; Atomic force microscopy

1. Introduction

Actin and myosin are key proteins in the regulation of muscle contraction and are found in a wide range of muscle and non-muscle cells. Myosin II forms bipolar filaments that interact with actin filaments to produce contraction and is implicated in the 'rounding up' of cells prior to cytokinesis and to drive post-mitotic cell spreading in highly motile cells (MacIver, 1996; Bloor and Kiehart, 2001). Upon addition of adenosine triphosphate (ATP), the actomyosin binding is loosened, which allows the sliding of the actin filaments. Upon hydrolization of ATP, the myosin heads stay attached to the actin filaments. While not generally thought of as an actin cross-linking protein, myosin II has been described as having this capability (Humphrey et al., 2002; Laevsky and Knecht, 2003; Smith et al., submitted for publication).

In this study, we first use the rheometer to examine the effect of myosin II on F-actin cross-linking, and then compare these findings with observations using atomic force microscopy. Previous rheological measurements by Humphrey et al. (2002) demonstrated that at a G-actin to myosin II molar ratio r_{AM}=257, myosin acts in the presence of 0.5 mM adenosine diphosphate (ADP) as cross-linker. The elastic (G') component of the solution was increased on average by a factor of 6, whilst the viscous (G'') component was slightly decreased. The authors described this phenomenon as the 'gelation' of the actin solution (Wachsstock et al., 1994); thus at the cellular level, this must be viewed as the stabilization of the cell cortex during cell motility.

2. Materials and methods

2.1. Protein preparation

Myosin was essentially prepared as described by Isenberg (1995). Actin was purified according to the procedure of Isenberg (1995) with an additional gel

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filtration step, as suggested by MacLean-Fletcher and Pollard (1980), using a Sephacryl S-300 column. The protein concentration was determined using $E_{290nm}=26{,}460\ M^{-1}\ cm^{-1}$. The G-actin peak fractions at 1–2 mg ml^{-1} were sterile-filtered and stored in G-buffer at 2 mM Tris/HCl, pH 7.5, 0.2 mM $CaCl_2$, 0.2 mM DTT, and 4 °C. Polymerization was started by adding 100 mM KCl, 2 mM $MgCl_2$ and 0.5 mM Na_2ATP at 20 °C to the G-actin solution. All reagents were supplied by Boston Bioproducts Inc, Worcester, MA 01604 (http://www.bostonbioproducts.com).

2.2. Rotation disc rheometer

The apparatus has been described in detail by Müller et al. (1991). The dynamic storage modulus $G'(\omega)$ and the loss modulus $G''(\omega)$ were measured between 4×10^{-3} and 4×10^{1} Hz. In brief, the rheometer consists of a cylindrical glass cuvette with an inner diameter of 15 mm and a volume of ~1.5 ml. The base of the cuvette is mounted in an aluminum thermostated holder. A glass disc with a diameter of 8 mm is placed on the surface of the viscoelastic solution. On top of the disc a magnet and deflection mirror with the dimension of $0.5\times0.5\times1.5\ mm^3$ and $1.5\times2.0\ mm^2$ are mounted, respectively. The plane of the deflection mirror forms an angle of 45° with the horizontal line. The glass cuvette is surrounded by two perpendicularly oriented magnetic coils. One of these serves to fix the orientation of the disc and the other (the deflection coils) to apply a shear force to the viscoelastic solution. When measuring the dynamic moduli $G'(\omega)$ and $G''(\omega)$ the deflection coils are driven by an oscillatory voltage of the frequency ω. The magnetic coils and the measuring cuvette were placed into a μ-metal chamber to shield off external magnetic fields. The rotational amplitude, $\alpha(t)$ of the disc is analyzed as follows: the beam of a He–Ne laser incident light—in a parallel direction along the rotational axis of the disc—is horizontally deflected by the mirror mounted on the disc. Its horizontal orientation is recorded by a position-sensitive photodiode, amplified and evaluated by computer. The actin solution in the measuring cuvette is covered by a phospholipid monolayer (dimyristoylphosphatidylcholine) which is essential to avoid the gelation of actin, owing to its denaturing at the air–water interface. This ensures good mechanical contact to the glass disc when covered by a monolayer of octadecyltrichlorosilane. Protein mixtures of ~400 μl total volume were polymerized for 1 h in the rheometer prior to experimentation.

2.3. Atomic force microscopy (AFM)

A Model 3000 AFM (Digital Instruments, St. Barbara, CA) attached to a Nanoscope IIIa controller with an electronics extender box was used for the present studies. The tapping mode configuration, characterized by the use of an oscillating tip, which intermittently touches the surface of the sample, was applied, since it is the least perturbating approach when used at the lowest possible energy delivery to the surface. Images were obtained with oxide-sharpened silicon-nitride tips (DNP-S; Digital Instruments) mounted on thick (21 nm) and short (100 nm) cantilevers, unless otherwise stated. The cantilevers had nominal spring constants of 0.58 N m^{-1}, and were thermally equilibrated before imaging. Drive frequencies obtained with these cantilevers were typically in the range of 7.9–8.2 kHz. Samples were deposited onto freshly cleaved mica (New York Mica Co., New York) discs mounted onto magnetic circular pucks. No filtering or data modification were performed (Chasan et al., 2002).

3. Results and discussion

3.1. Frequency-dependent measurements

The goal of this study was to use the rheometer to determine the actin filament stiffness in the presence/absence of myosin and to verify findings by atomic force microcopy. We performed the first experiments at an actin/myosin molar ratio, $r_{AM}=1{:}10$ and actin concentration, $c_A=0.5$ mg/ml in the presence of 0.5 mM ADP, which turned actin filaments into a network of high elasticity not measurable by the rheometer (data not shown). To carry out meaningful measurements under defined conditions, we lowered the actin concentration to $c_A=0.4\ mg\ ml^{-1}$ and changed the myosin/actin molar ratio to $r_{AM}=1{:}200$. Using these conditions, we observed a marked increase in the storage modulus (elastic part, G') compared to pure F-actin (Fig. 1a,b, —⊖—). When comparing this observation with F-actin in the presence of natural cross-linkers like alpha-actinin (Tempel et al., 1996) or filamin (ABP-280) (Goldmann et al., 1997), much higher molar ratios are needed to accomplish the same effect. Further, we noticed that the values for $G'(\omega)$ in the presence of myosin are more scattered than those of pure F-actin (Fig. 1b).

A closer look at Fig. 1a,b shows that the loss modulus (viscous part, G'', - -⊡- -) of a pure F-actin solution continuously increases at rising frequency, whilst the myosin containing probe shows a minimum at $(\omega)\sim0.03$ Hz. This plateau region is usually expected from cross-linked solutions at much lower frequencies compared to non-cross-linked polymers as cross-linked polymers normally do not show any flow behavior, which allows the plateau to stretch to lower frequencies. As this usually applies only to homogeneously cross-linked polymers, one can speculate that the differently

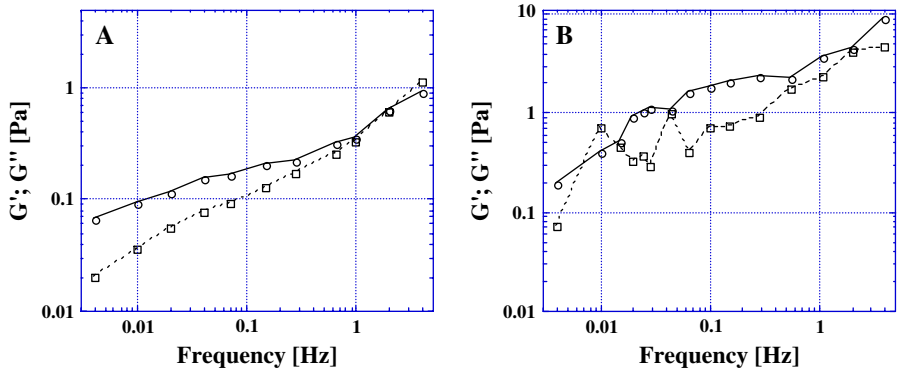

Fig. 1. Viscoelastic moduli, G′(——○——, storage modulus) and G′′(– –□– –, loss modulus) of a 0.4 mg/ml F-actin solution in the absence (A) and presence (B) of myosin II at a molar ratio r_{MA} of 1:200. Actin was polymerized for 20 min in 2 mM Tris/HCl, pH 7.5, 0.2 mM $CaCl_2$, 0.2 mM DTT, 100 mM KCl, 2 mM $MgCl_2$, and 0.5 mM Na_2ATP at 20 °C before adding myosin II. The data are an average of 3 different experiments.

observed curve structure is due to the heterogeneous structure of the network. Thus, we observed irregular binding of myosin heads (S1) to actin filaments from images taken by the atomic force microscope (Fig. 2), which partly confirm this assumption. However, it is also conceivable that the flow of highly concentrated regions starts below a certain border frequency, when regions of different actin concentration meet, which would mark the lower end of the plateau. This process is not possible in homogenous, non-cross-linked actin solutions and is normally not detectable.

It has been reported that the ability of the cytoskeleton to deform and reform is a crucial aspect of many cellular processes such as cell motility, cell division, and cell shape control (Chen et al., 1997). When cells migrate, they continuously change their cytoskeleton and preventing these cytoskeletal changes by affecting actin polymerization, myosin phosphorylation inhibits cell motility (Wilson et al., 1991). Inhibiting actin-myosin II interactions seems to decrease cytoskeletal stiffness. Cytoskeletal stiffness is also decreased in experiments where disruption of the actin filament has occurred. The implication of these observations is that decreasing actin–myosin II interaction releases the internal tension generated by these proteins that make the cell cytoskeleton less stiff (Bloor and Kiehart, 2001). Conversely, actin–myosin II interactions should also increase cell stiffness by generating tension within cells (Cai et al., 1998).

A unique feature of the protein networks in living cells is that they can generate their own force. Proteins such as myosin are an integral part of the cytoskeleton and have the capacity to convert the energy of ATP hydrolysis into directional movement. Myosin II can move actin filaments against each other, and depending on the orientation of the filaments and the way in which they are linked together, it can produce contraction, bending, extension, and stiffening. This is best studied in striated muscle cells. Contractile structures in non-muscle cells are less well defined in structure and correspondingly more difficult to handle, although myosin II is a major contributor of cortical tension (Polte et al., 2004).

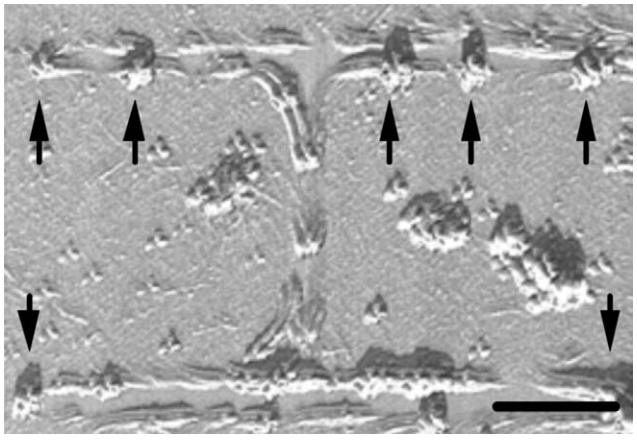

Fig. 2. The atomic force micrograph depicts polymerized actin (0.042 mg/ml) in the presence of myosin II (0.001 mg/ml). Buffer conditions: 100 mM KCl, 2 mM $MgCl_2$ and 0.5 mM Na_2ATP at 20 °C. The myosin heads clearly cross-link the actin filaments. Note: The image shows slight 'double-tipping' of the S1 head in the x-direction. Drive frequency 7.9-8.2 kHz. Bar: 1 μm.

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