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Counterion-induced actin ring formation

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Abstract Actin filaments form rings and loops when >20 mM divalent cations are added to very dilute solutions of phalloidin-stabilized filamentous actin (F-actin). Some rings consist of very long single actin filaments partially overlapping at their ends, and others are formed by small numbers of filaments associated laterally. In some cases, undulations of the rings are observed with amplitudes and dynamics similar to those of the thermal motions of single actin filaments. Lariat-shaped aggregates also co-exist with rings and rodlike bundles. These polyvalent cation-induced actin rings are analogous to the toroids of DNA formed by addition of polyvalent cations, but the much larger diameter of actin rings reflects the greater bending stiffness of F-actin. Actin rings can also be formed by addition of streptavidin to crosslink sparsely biotinylated F-actin at very low concentrations. The energy of bending in a ring, calculated from the persistence length of F-actin and the ring diameter, provides an estimate for the adhesion energy mediated by the multivalent counterions, or due to the streptavidin-biotin bonds, required to keep the ring closed.

Keywords Biopolymer · Polyelectrolyte · Attractive interaction · Cytoskeletal filaments · Cell motility

Introduction

The self-association of like-charged polymers caused by multivalent counterions is a commonly observed phenomenon for which a theoretical explanation is still an ongoing effort. In cases where a specific chemical bond between filaments, or a chemical crosslink due to the counterion, can be ruled out, a number of models based on spatial arrangement or fluctuations of counterions on the linear polyelectrolyte have been proposed to account for the attractive force. Most experimental tests of such models are derived from studies of double stranded DNA, and the general application of these models to other systems is limited. Some recent studies have emphasized that the polyelectrolyte properties of cytoskeletal polymers are similar to those of DNA, and predictions made from studies of DNA can be tested in these systems. A particularly striking form of counterion-mediated condensed DNA is toroids of single or multiple DNA molecules (Baeza et al. 1987). The condensed DNA toroids are preferentially formed when the DNA is too dilute to form large lateral aggregates, and the average diameter of the toroids is comparable to the persistence length of DNA. The same concepts applied to other charged filaments suggest that they too may form similar rings whose structures may differ from those of DNA toroids in a manner predictable by differences in linear charge density, filament diameter, bending stiffness, etc.

Actin is a highly expressed cytoskeletal protein that plays essential roles in the mechanical properties of eukaryotic cells, as well as with their division and locomotion properties (Condeelis 1993; Stossel 1993). Control of these functions depends on remodeling of
networks composed of the filamentous actin (F-actin), which undergoes dynamic assembly and disassembly, as well as crosslinking and lateral association both in vitro and in vivo (Alberts et al. 1994; Carlier et al. 1994; Kawamura and Maruyama 1970; Oosawa 1993). F-actin is a linear protein filament with a diameter of 8 nm, a persistence length approximately 17 μm (Gittes et al. 1993; Isambert et al. 1995; Ott et al. 1993), and a nominal charge density of 11 negative fundamental charges per monomer subunit, or −4e− per nm along the filament axis (Tang and Janmey 1996). Previous studies have established the polyelectrolyte nature of F-actin, and consequently the lateral association of F-actin to form large bundles induced by polyvalent counterions (Tang and Janmey 1996; Tang et al. 1997; Xian et al. 1999). This report documents the formation of actin rings and lariat-shaped aggregates induced by a simple divalent metal ion, Mg2+. Microscopic observations of size, shape, and undulations of the actin rings are related to the measured stiffness of F-actin. Actin rings are also formed using streptavidin to crosslink sparsely biotinylated F-actin, allowing a comparison between the lateral adhesive force due to electrostatic attraction and that due to a specific type of biochemical crosslink.

**Materials and methods**

Actin was purified according to the method described by Spudich and Watt (1971). The monomeric globular (G)-actin was kept in a nonpolymerizing buffer containing 4 mM Heps at pH 7.5, 0.2 mM CaCl2, 0.5 mM adenosine triphosphate (ATP), 0.2 mM dithiothreitol (DTT), and 0.5 mM sodium azide (NaN3). F-actin was polymerized from G-actin by 150 mM KCl. Biotinylated actin was purchased from Cytoskeleton (Denver, Co., USA). Streptavidin was purchased from Molecular Probes (Eugene, Ore., USA). All chemicals were of analytical grade, supplied by either Sigma (St. Louis, Mo., USA) or ICN Pharmaceuticals (Costa Mesa, Calif., USA).

For microscopic observation, TRITC-labeled phalloidin was added to the polymerized actin at a 1:1 molar ratio. Then a small aliquot of the stock F-actin solution was diluted to nanomolar concentrations, followed by adding MgCl2 to 50 mM. Alternatively, biotinylated G-actin was mixed with unlabeled G-actin at a 1:4 molar ratio, and the mixture was polymerized as above with TRITC-labeled phalloidin. Upon dilution to nanomolar protein concentration so that the solution contained only about one F-actin per (10 μm)3 volume, streptavidin, a tetrafunctional ligand for biotin, was added at a ratio of 1 avidin per 2 biotin in order to crosslink F-actin, with the expectation that intra-filament links would form looped structures or rings.

Fluorescently labeled F-actin was visualized on a Nikon Diaphot 300 inverted microscope equipped with epifluorescence optics and a Nikon PlanApo 60× (NA 1.40) or PlanFluor 100× (NA 1.30) objective. The motions of the filaments were captured to a DAGE-MTI silicon intensified target (SIT) camera (DAGE-MTI, Michigan, IL) through a 2× optical coupler, and digitized to a Macintosh computer (Apple Computer, Cupertino, Calif.) through a Scion AG-5 video capture board (Scion Corporation, Frederick, MD). The images were typically captured at 15 frames/s for up to 1000 frames. Motions of the labeled filaments were simultaneously recorded to VHS videotape, which allowed alternative image capturing and additional analysis. Filaments that diffused out of the focal plane were not used in the analysis.

For each digitized frame, the fluorescent filament image was traced using an automated algorithm as described below, and the coordinates saved to a file. The algorithm design was based on the point spread function of the microscope.

The intensity profile of a fluorescent filament is well approximated by a Gaussian function. The filament tracing algorithm begins by slicing through the local intensity profile and finds the best fit to a quadratic function. A quadratic function is used to compute the local intensity profile because its computation is simpler and faster than a Gaussian fit and it approximates the local profile near the intensity maximum. The quadratic function is computed over a number of orientations with respect to the filament. The steepest quadratic with an acceptable correlation coefficient is chosen to be the local intensity profile of the filament. In this way the algorithm determines both the actual orientation of the filament and the local intensity maximum, i.e. the approximate location of the filament in space. The algorithm proceeds by advancing along the calculated orientation of the filament and re-calculating the local quadratic functions. Through this iterative scheme the filament contour can be determined. When the algorithm reaches a region that overlaps the starting point, the tracing of the ring is complete.

Analyzing real fluorescent filaments can be difficult owing to the presence of noise, both electronic and fluorescent. Before being traced, each video frame undergoes a pre-processing step using a log-Gaussian filter with spatial frequency optimized for the width of the fluorescent filament signal to enhance the filament intensity with respect to the background noise. Ring filament contour coordinates are analyzed to determine center of mass, which is then set as the origin of azimuthal coordinates.

**Results**

Formation of actin rings and other structures

Actin filaments exhibit self-attractive interactions after addition of sufficient divalent metal ions, such as 50 mM MgCl2, as used in this study. When MgCl2 was added into a very dilute F-actin solution in the nanomolar range, we occasionally observed that a subset of actin filaments assembled into various structures distinct from rodlike bundles, such as branched lateral aggregates and loops with or without additional tail(s) (Fig. 1). In rare cases, the two ends of a single filament adhered to each other to form an actin ring with fluorescence intensity through much of its contour comparable to that of a single labeled filament. The upper left image of Fig. 1 shows an example of an actin ring formed by a single long filament of 15–20 μm contour length.

Actin rings formed by a number of filaments are often kinked, and the kinks appear to be where some filaments end, discernible by a marked difference in fluorescence intensity over a particular kink. Also, segments with relatively higher fluorescence intensities appear to bear less bending, another indication of more filaments packed in the bundle.

Undulation of actin rings in solution

Although most of the images shown in Fig. 1 represent structures bound to the surface of glass slides, various looped structures were also observed in solution. The closed rings adherent to the glass substrate were likely formed in solution, although the strong anchoring effect