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Polycation induced actin bundles

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ABSTRACT

Three polycations, polylysine, the polyamine spermine and the polycationic protein lysozyme were used to study the formation, structure, ionic strength sensitivity and dissociation of polycation-induced actin bundles. Bundles form fast, simultaneously with the polymerization of MgATP-G-actins, upon the addition of polycations to solutions of actins at low ionic strength conditions. This indicates that nuclei and/or nascent filaments bundle due to attractive, electrostatic effect of polycations and the neutralization of repulsive interactions of negative charges on actin. The attractive forces between the filaments are strong, as shown by the low (in nanomolar range) critical concentration of their bundling at low ionic strength. These bundles are sensitive to ionic strength and disassemble partially in 100 mM NaCl, but both the dissociation and ionic strength sensitivity can be countered by higher polycation concentrations. Cys374 residues of actin monomers residing on neighboring filaments in the bundles can be cross-linked by the short span (5.4 Å) MTS-1 (1,1-Methanedyl Bismethanethiosulfonate) cross-linker, which indicates a tight packing of filaments in the bundles. The interfilament cross-links, which connect monomers located on oppositely oriented filaments, prevent disassembly of bundles at high ionic strength. Cofilin and the polysaccharide polyanion heparin disassemble lysozyme induced actin bundles more effectively than the polylysine-induced bundles. The actin-lysozyme bundles are pathologically significant as both proteins are found in the pulmonary airways of cystic fibrosis patients. Their bundles contribute to the formation of viscous mucus, which is the main cause of breathing difficulties and eventual death in this disorder.

1. Introduction

Cells contain a number of dynamic, higher order structures, including bundles of actin filaments. These structures have indispensable role in cell physiology, in the formation of cytoskeleton, cell division, motility, adhesion and signaling, etc. Actin bundles may form via cross-linking of individual actin filaments with specific actin-bundling proteins [1] or by attracting the negatively charged filaments to the polycations which "bridge" between the filaments. The group of bivalent cross-linking proteins includes fimbrin, α-actinin, spectrin, fascin, filamin and others. These proteins [1] have two discrete F-actin binding sites and form tightly bound parallel filaments [2,3].

Polycations, including polycationic proteins or peptides and natural and synthetic polyamines, polymerize actin and induce bundle formation via non-specific electrostatic interactions, by eliminating repulsion between individual G-actin molecules or between actin filaments [4]. Calponin [5,6], lysozyme [7] MARCKS [8,9], ENA/VASP [10], fesselin [11,12] and myelin basic protein [13] are examples of such polycationic proteins. The natural polyamines spermine and spermidine [14,15] and the synthetic polyamine polylysine are known to polymerize and bundle actin [16]. The bundling effect of polycations is known to decrease with increasing ionic strength [17], but the kinetics of bundle formation, cross link formation between the filaments in the bundle, interaction of polycation-induced bundles with actin binding and severing proteins remain poorly understood or uncharacterized.

Three polycations, lysozyme, spermine and polylysine were chosen in this study to investigate the various aspects of polycation-induced actin-bundling. Lysozyme is an antibacterial polycationic protein with 9 net positive charges [7]. It is abundant in the airways of cystic fibrosis patients [18], where it forms bundles with actin [19]. These bundles contribute to the accumulation of sputum, a viscous mucus — the primary cause of bacterial infections and death in this disease [20]. The viscosity of sputum was found to be significantly decreased by the actin severing protein gelsolin [21] and by polyanions [22]. The natural polyamine spermine, which is a small tetravalent cation, is present at millimolar concentrations in proliferating cells [23], and is believed to play a significant role in cell cycle, apoptosis, and signal transduction [24,25]. The synthetic polyamine polylysine is one of the most efficient polymerizing and bundling agents and has been used as model compound in a number of studies on polycation formed actin bundles [4,26,27].

In this study, we examined the formation and structure of actin bundles induced by these three polycations using light scattering, low speed sedimentation and cross-linking methods. We found that bundles,

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which form simultaneously with actin polymerization, have a very low critical concentration at low ionic strength. The bundles are ionic strength sensitive and those induced by low millimolar concentrations of spermine or low micromolar concentrations of lysozyme or polylysine disassemble fast in the presence of 100 mM NaCl. Their ionic strength sensitivity decreases with the increase of polycation concentration. The bundles are partially disassembled by the actin filament severing protein cofilin [28], whose unbundling activity is significantly enhanced by the polysaccharide polyanion heparin. The tightly packed polycation-induced bundles contain oppositely oriented actin filaments, as indicated by the interfilament cross-links between Cys-374 residues by the short span (5.4 Å) MTS-1 cross-linking reagent.

2. Materials and methods

2.1. Materials

N-(1-pyrene)maleimide was obtained from Molecular Probes (Eugene, OR). Hen lysozyme, ATP, ADP, poly-L-lysine (MW 4000), heparin (unfractionated), dithiotreitol (DTT), N-ethylmaleimide (NEM), spermine and EGTA were purchased from Sigma Chemical Co. (St Louis, MO). 1,1-methaediyl bismethanethiosulfonate (MTS-1), was obtained from Toronto Research Chemicals Inc., North York (Ontario, Canada). Bacterial transglutaminase and N-(4-azido-2-nitrophenyl) putrescine (ANP) were generous gifts from Dr. G. Hegyi (Eotvos Lorand University, Budapest, Hungary).

2.2. Preparation of actin

CaATP-G-actin was prepared from the back and leg muscles of rabbit by the method of Spudich and Watt [29] and stored in G-buffer containing 5.0 mM Tris–HCl, 0.2 mM $CaCl_2$, 0.2 mM ATP, and 0.5 mM β-mercaptoethanol, pH 8.0 (CaATP-G-buffer). MgATP-G-actin was obtained by incubating CaATP-G-actin with 0.2 mM EGTA and 0.1 mM $MgCl_2$ at room temperature for 5 min. MgATP-G-actin ($\leq$10 μM) was used within 2 hours from the preparation. MgATP-G-actin was diluted for further treatments in MgATP-G-buffer containing 5 mM Tris–HCl, 0.1 mM $MgCl_2$, 0.2 mM EGTA, 0.2 mM ATP and 0.5 mM DTT, pH 8.0. Mg-F-actin was polymerized from Mg-ATP-G-actin by addition of 2 mM $MgCl_2$. The concentration of unlabeled skeletal muscle α-G-actins was determined spectrophotometrically using the extinction coefficients $E^{1\%}_{290} = 11.5\ cm^{-1}$. (The optical density of actin was measured in the presence of 0.5 M NaOH, which shifts the maximum of absorbance from 280 nm to 290 nm). Molecular masses of skeletal actin, yeast cofilin and hen lysozyme were assumed to be 42 kDa, 15.9 kDa and 14.3 kDa, respectively.

2.3. Cofilin preparation

Yeast cofilin was prepared as previously described [30] with minor modifications. Briefly, wild-type yeast cofilin was expressed in *Escherichia coli* BL21(DE3) cells under the T7-promoter (pBAT4 plasmid). Cells were grown to a density of 0.6 OD units, induced with 0.4 mM IPTG, harvested by centrifugation, resuspended in 20 mM Tris–HCl, pH 7.5, and lysed by sonication. The cell lysate was clarified by centrifugation, the supernatant applied to a QAE-52 column (Pharmacia Biotech) equilibrated with 20 mM Tris–HCl, pH 7.5 and the column developed with a linear 0–0.5 M NaCl gradient. Peak fractions containing cofilin were pooled, concentrated, and applied to a Sephacryl S300 gel-filtration column (Pharmacia Biotech), equilibrated with 10 mM Tris–HCl, 50 mM NaCl, pH 7.5. The peak fractions were pooled and polished by MonoQ ion-exchange chromatography using a buffer system similar to that described for the QAE-52 chromatography.

2.4. Chemical modification

Labeling of Mg-F-actin at Cys-374 with pyrene maleimide was carried out according to Kouyama and Mihashi [31] with some modifications. CaATP-G-actin was filtered through a PD-10 column equilibrated with β-mercaptoethanol free Ca-ATP-G-buffer. After filtration, actin (1.0 mg/ml) was polymerized by 2.0 mM $MgCl_2$ and 100 mM KCl at room temperature for 30 min, and reacted with pyrene maleimide (16 μg/ml) on ice, for 1 hour. The reaction was terminated with 1.0 mM DTT. The labeled F-actin was centrifuged at 38 K rpm for 2 hours, then the pellet was resuspended in Ca-ATP-G-buffer and depolymerized for over 36 h at 4 °C. Finally, actin was centrifuged again at 38 K rpm for 2 hours. The supernatant contained the purified pyrene-labeled CaATP-G-actin. The concentration of modified actin was determined by the procedure of Bradford [32] using unmodified actin as a standard. The extent of labeling, which was measured by using pyrene extinction coefficient $E_{344\ nm} = 22000\ cm^{-1}\ M^{-1}$, was ~100%. G-actin was labeled by ANP, polymerized by 2 mM $MgCl_2$ and photo cross-linked according to Hegyi et al. [33]. The extent of labeling was calculated by extinction coefficient $E_{470} = 5400\ cm^{-1}\ M^{-1}$ of ANP.

2.5. Covalent cross-linking

Cross-linking of two actin monomers between Cys-374 residues at the C-terminus was carried out with the MTS-1 disulfide reagent. This reagent has a cross-linking span of 5.4 Å and has been used before for intramolecular cross-linking of actin cysteine residues [34]. Prior to the cross-linking reaction, dithiothreitol was removed from CaATP-G-actin over a PD-10 column equilibrated with β-mercaptoethanol free Ca-ATP-G-buffer. Then CaATP-G-actin was transformed to MgATP-G-actin, polymerized by 2 mM $MgCl_2$ and finally bundled by polycations. MTS-1 was added to bundles of Mg-F-actin at 0.75:1.0 molar ratios to actin monomers. The cross-linking reaction was stopped 30–60 s after MTS-1 addition by blocking the free SH groups with 1.0 mM NEM. The cross-linked samples were analyzed by 12% SDS-PAGE and quantitatively evaluated by densitometry.

2.6. Fluorescence and light scattering measurements

The time course of pyrene-labeled actin polymerization was monitored by measuring fluorescence increase (with 365 nm excitation and 386 nm emission wavelengths) in a PTI spectrofluorometer (Photon Technology Industries, South Brunswick, NJ). The time course of light scattering changes was also measured in a PTI spectrofluorometer or in an Applied Photophysics stopped-flow apparatus (Leatherhead, Surrey, UK), with both excitation and emission wavelengths adjusted to 450 nm. All fluorescence and light scattering measurements were carried out at 22 °C.

2.7. Monitoring bundling by low speed sedimentation

After the addition of polycations, actin samples were centrifuged at 20800 rcf for 8 min in an Eppendorf centrifuge. The supernatants were analyzed by SDS-PAGE. The measurements were carried out at 22 °C.

2.8. Analysis of the kinetics of assembly and disassembly of bundles

GraphPad Prism software was used for the analysis. The explicit forms of the equations used are the following: Two phase exponential association: $Y + Ymax_1\ (1 - \exp(-K_1\ X)) + Ymax_2\ (1 - \exp(-K_2\ X))$; one phase exponential decay: $Y = Span\ (\exp(-K\ X) + Plateau$.

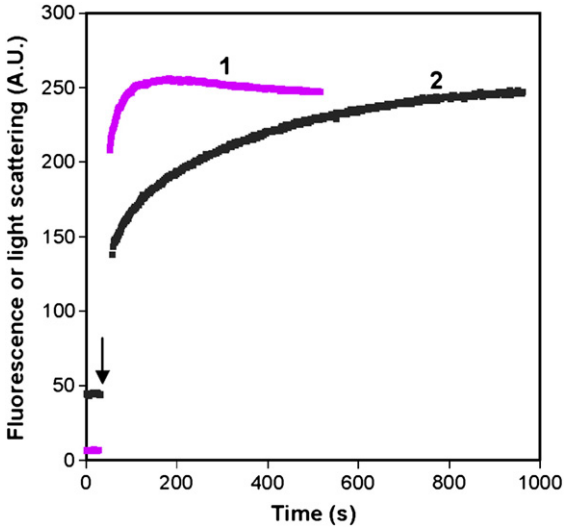


Fig. 1. Polymerization and bundle formation of 2 μM pyrene-labeled MgATP-G-actin upon the addition of 2 μM lysozyme at low ionic strength. Polymerization and bundling were monitored by following pyrene fluorescence and light scattering changes, respectively, as described in Materials and methods. Curve 1, bundling; curve 2, polymerization. Addition of 2 μM lysozyme is shown by an arrow.

3. Results

3.1. Bundle formation during polymerization of MgATP-G-actin by polycations

Bundles are formed when G-actin is polymerized at low ionic strength by polycations [35]. Bundle formation can be followed either by low speed sedimentation [15] or by the increase in light scattering [4]. We compared the course of MgATP-G-actin polymerization and bundling by following these processes via increases in pyrene fluorescence and light scattering, respectively. This is feasible because pyrene fluorescence measures actins polymerization but is essentially insensitive to its bundling (fluorescence increases ~10-fold upon polymerization but only ~10% upon bundling of F-actin), while the light scattering increase is relatively small upon actin polymerization (~2.8-fold) compared to the large change (~11-fold) caused by the bundling of actin filaments. Filament bundling by polylysine, lysozyme and spermine proceeds approximately at a similar or somewhat faster rate than filament elongation (Fig. 1; only lysozyme data is shown). These results indicate that in the polycation-induced polymerization even the nascent or very short filaments assemble immediately following the nucleation step, with the elongation of the filaments continuing also after their bundling.

3.1.1. Polycation induced bundle formation from MgF-actin

Bundle formation upon the addition of polycations to preformed MgF-actin filaments (Fig. 2a and b) is very fast, much faster than their formation from MgATP-G-actin (Fig. 1), at low ionic strength conditions. The bundles formed at relatively low concentrations of polycations are sensitive to the ionic strength and disassemble “unbundle” very fast upon the addition of 100 mM NaCl. The formation of bundles from 2 μM Mg-F-actin upon addition of 4 μM polylysine (Fig. 2b) appears biphasic and could be fitted by two kinetic steps with 0.83 and 0.052 s^{-1} rate

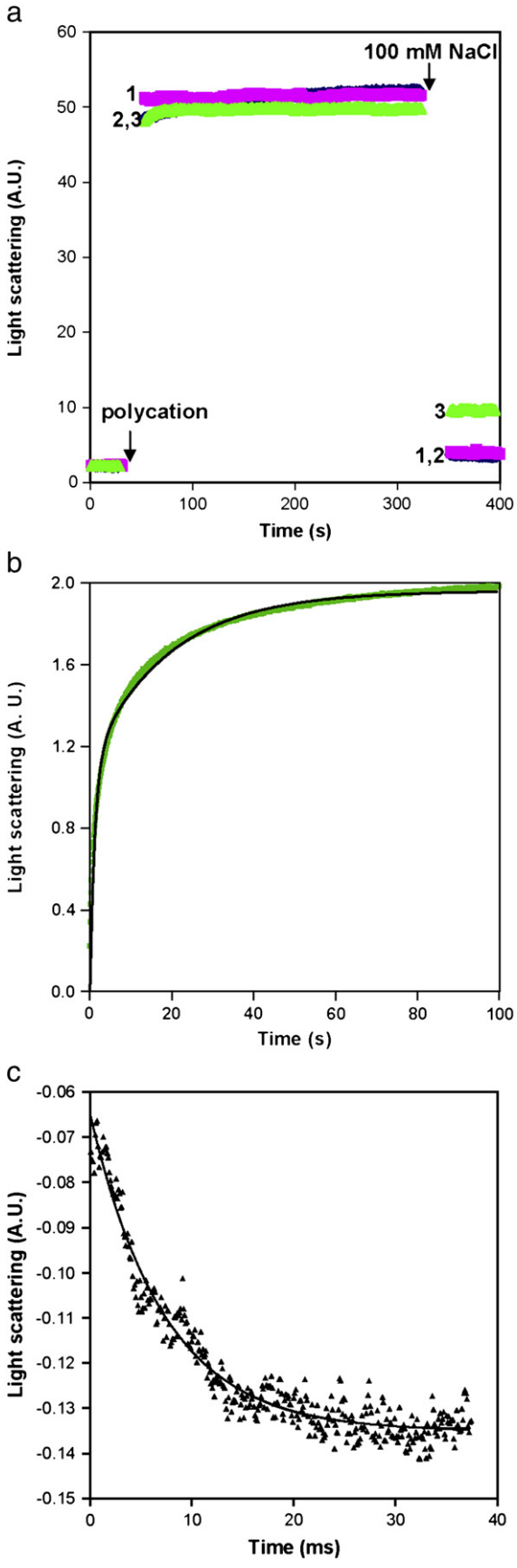


Fig. 2. Bundle formation of Mg-F-actin by polycations and dissolution of Mg-F-actin bundles by 100 mM NaCl. Bundling was monitored by light scattering changes. (a), Bundling of 2 μM MgF-actin by 4 μM lysozyme (1, pink), 0.5 mM spermine (2, dark blue) and 4 μM polylysine (3, green); unbundling by 100 mM NaCl. Addition of polycations and NaCl is shown by arrows. Light scattering change was followed in a PTI fluorometer. (b), Bundling of 2 μM MgF-actin by 4 μM polylysine. Green curve, fluorometer tracing; black curve, fitting to a two phase association. Light scattering was measured in a stopped flow fluorometer. (c), 100 mM NaCl dissolves bundles of 2 μM MgF-actin formed by 4 μM polylysine. Triangles, fluorometer readings; curve, fitting to a single exponential decay. Light scattering was measured in a stopped flow apparatus as described in Materials and methods.

constants. The unbundling of the fresh polylysine-induced bundles by 100 mM NaCl was much faster than their formation and could be described by a single exponential decay, with a rate constant of 140 s^{-1} (Fig. 2c).

The extent of bundling of actin filaments by polycations, as measured by low speed sedimentation experiments, was dependent both on the nature and the concentration of the polycations, on the ionic strength of the solution (Fig. 3a). At physiological ionic strength (100 mM NaCl), about one order of magnitude higher concentration of polycation was needed for the bundling than at low ionic strength. Lysozyme was the most efficient bundling agent at low while polylysine at physiological ionic strength. Half maximal bundling of 10 μM Mg-F-actin was obtained at low ionic strength at 3.1, 6.0 and 310 μM while at physiological ionic strength at 30, 20 and 3650 μM for lysozyme, polylysine and spermine, respectively. These results clearly show the competing effect of ionic strength and polycation concentration on bundling.

We studied next the F-actin concentration dependence of bundling at physiological ionic strength conditions. In these experiments 10–50 μM lysozyme or polylysine were added to 8–60 μM Mg-F-actin in the presence of 100 mM NaCl and the degree of bundling was measured by low speed centrifugation (Fig. 3b). The concentration of polycation needed for bundle formation increased with the concentration of Mg-F-actin, however, the dependence of bundling on actin concentration was less pronounced than that on the polycation concentration.

We measured the critical concentration of Mg-F-actin bundling at low ionic strength using polylysine as a model polycation. To this end, polylysine (6 μM) was added to 100 nM Mg-F-actin, and serial dilutions were carried out after maturing the bundles. Each sample was centrifuged at low speed and the protein content in the pellet was determined yielding the critical concentration for bundling of 1.8 nM actins. The extremely low critical concentration of the polylysine-bundled actin indicates that the affinity between actin filaments is very high in the presence of polycation counter-ions.

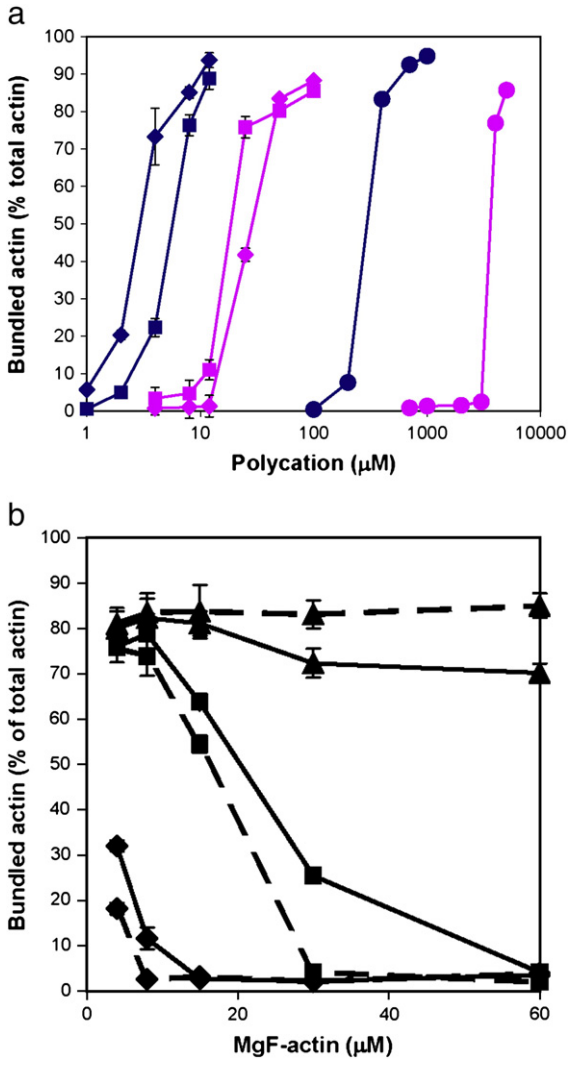


Fig. 3. Bundling of Mg-F-actin by polycations in the presence and absence of 100 mM NaCl. Actin bundling was monitored by low speed sedimentation as described in Materials and methods. Error bars correspond to mean errors from three independent experiments. (a), Effects of polycation concentration and ionic strength on the bundling. Diamonds, lysozyme; squares, polylysine; full circles, spermine. Bundling in the presence and absence of 100 mM NaCl is shown in pink and dark blue, respectively. (b) Effect of F-actin concentration on its bundling. Solid line, polylysine; dashed line, lysozyme. Polycation concentration is shown by symbols: diamonds, 10 μM; squares 25 μM; triangles, 50 μM polycation.

3.1.2. Cofilin disassembles polycation-induced actin bundles

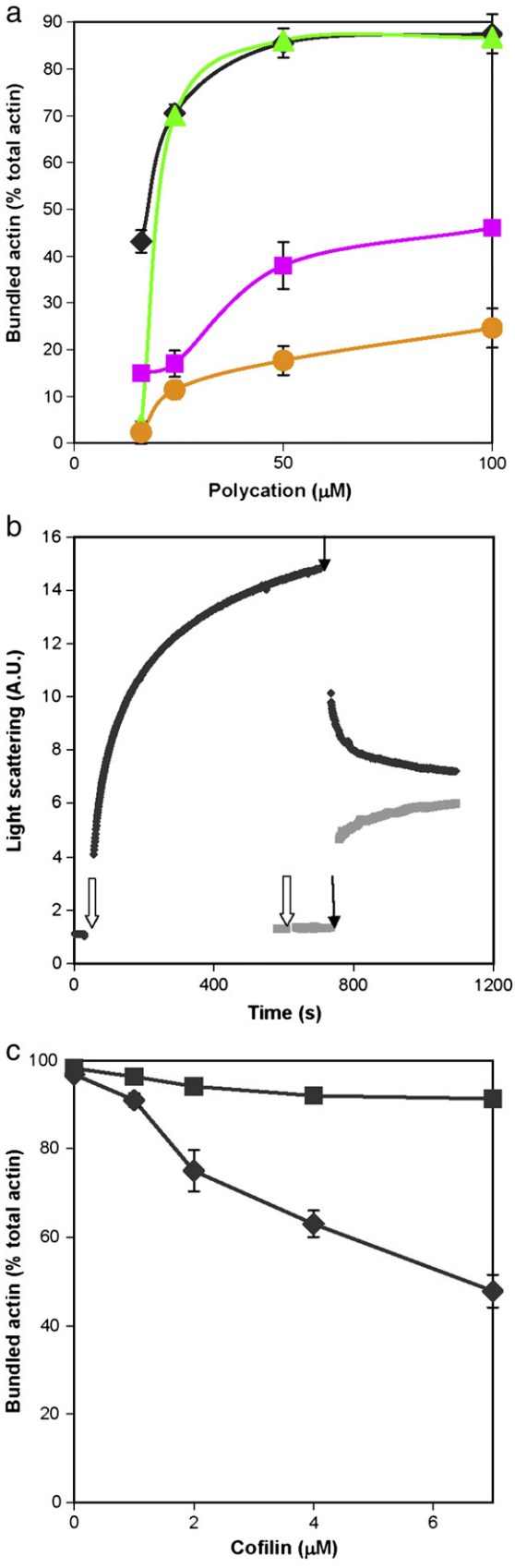
Actin filament bundles are major contributors to the viscosity of sputum in the airways of cystic fibrosis patients. Gelsolin, an actin filament severing protein was found to significantly reduce the viscosity of sputum [21]. Here, we studied the effect of another actin filament severing protein, cofilin [28], on the stability of polycation-induced bundles. Addition of 8 μM cofilin to 8 μM lysozyme- or polylysine-induced bundles at physiological ionic strength (Fig. 4a) significantly reduced, but did not eliminate completely the bundled actin. The degree of disassembly decreased with the increase of polycation concentration used for bundling indicating competition between cofilin and the polycations. The unbundling effect of cofilin was stronger on lysozyme- than on polylysine-induced bundles. The disassembly of bundles was rather fast, it took about 1 min upon the addition of cofilin to reach new equilibrium in light scattering measurements (Fig. 4b). Essentially the same amount of actin remained bundled after reaching equilibrium when cofilin was added to lysozyme bundled actin or lysozyme was added to F-actin, which already contained cofilin (Fig. 4b). Finally we checked the role of severing in the disassembly by inhibiting the former through ANP-cross-linking of actin bundles (Fig. 4c). ANP covalently cross-links protomers along the long pitch helix of the actin filaments [33], decreasing their severing and, therefore, completely inhibiting the unbundling by cofilin.

In order to enhance the disassembly of the bundles, we added heparin together with cofilin to the system (Fig. 5). Heparin, which is a polysaccharide polyanion, has similar destabilizing effect on cystic fibrosis sputum [36] as the anionic polyamino acids [22]. We added increasing concentration of heparin to lysozyme-bundled actin filaments in the absence and presence of cofilin (Fig. 5). Heparin also dissociates F-actin bundles, however, when cofilin and heparin were added together the extent of disassembly increased significantly. Low concentration (0.3 mg/ml) of heparin, which alone has only a slight unbundling effect, increased the cofilin induced disassembly of bundles by 50%.

3.1.3. Polycation-induced bundles are stabilized by cross-linking "interfilament antiparallel dimers"

It has been proposed before that polycation-induced actin bundles contain antiparallel filaments [35]. Such oppositely oriented filaments in the bundle should position a fraction of actin protomers in these filaments in a spatial arrangement similar to that found in antiparallel dimers, except that in the present case these would be interfilament (proximity) antiparallel dimers (IAP). The presence of such IAP was detected in polylysine-bundled Mg-F-actin by MTS-1, which cross-linked Cys-374 residues from two actin protomers, but no IAP (or AP) dimer was found in free, unbundled Mg-F-actin filaments [35]. Here, we show the presence of IAP dimers in filament bundles induced by all the three polycations used in this study (Fig. 6). The amount of IAP dimers increased with the increasing polycation concentration. The highest amount of IAP dimer was found in bundles induced by polylysine (maximum 15.6% of total

actin), less in spermine-induced (maximum 11.6% of total actin) and much less in lysozyme-induced bundles (maximum 2.7% of total actin). The relatively low amount of IAP dimer in lysozyme-bundled Mg-F-actin



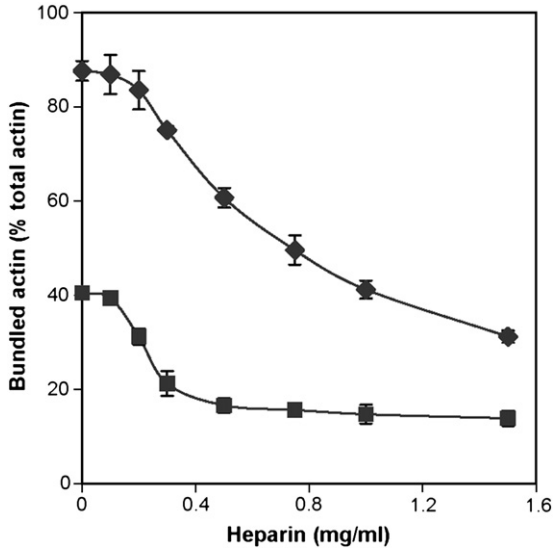


Fig. 5. Effect of heparin on the dissolution of the bundles of 6 μM Mg-F-actin by 6 μM cofilin. Mg-F-actin was bundled by 50 μM lysozyme in the presence of 100 mM NaCl. Unbundling was followed by low speed sedimentation as described in Materials and methods. Diamonds, cofilin absent; squares, cofilin present. Error bars correspond to mean errors from three independent experiments.

is probably due to the intercalation of lysozyme between the actin filaments [19], which may increase the distance between the filaments and inhibits the formation of IAP dimers.

The effect of cross-linking AP dimers on the stability of bundles is examined in Fig. 7. The bundles of 4 μM Mg-F-actin formed by lysozyme at low ionic strength were disassembled by 100 mM NaCl (as in Fig. 2a). However, after cross-linking these bundles persisted in the presence of 100 mM NaCl. The reversal of cross-links with DTT leads to the dissolution of bundles by 100 mM NaCl, analogous to the disassembly of uncross-linked (by MTS-1) bundles (similar results – not shown – were obtained also with polylysine and spermine induced bundles). Both cross-linked and uncross-linked lysozyme-induced bundles contained lysozyme in addition to actin, but lysozyme disappeared from the cross-linked bundles after the addition of NaCl (Fig. 7). This suggests that at physiological ionic strength the affinity between actin and lysozyme is mainly electrostatic.

4. Discussion

Polycations efficiently polymerize and bundle actin. The polycation-induced bundles could be important elements of the cellular actin dynamics, participating in the formation of cytoskeletal structures. We examined the kinetics of polymerization and bundle formation following the addition of polycations to MgATP-G-actin at low ionic strength. The two processes proceed simultaneously; however, the initial rate of bundling appeared faster than that of the filament elongation. This shows

Fig. 4. Dissolution of polycation induced actin bundles by cofilin. (a), 8 μM cofilin was added to 8 μM Mg-F-actin bundled by 16–100 μM lysozyme or polylysine in the presence of 100 mM NaCl. Bundling was monitored by low speed sedimentation as described in Materials and methods. Diamonds, polylysine without cofilin; squares, polylysine and cofilin; triangles, lysozyme without cofilin; full circles, lysozyme and cofilin. (b) Effect of 8 μM cofilin on the light scattering of 8 μM Mg-F-actin bundled by 50 μM lysozyme (black trace) and the effect of 50 μM lysozyme on the scattering of 8 μM Mg-F-actin in the presence of 8 μM cofilin (half tone trace). Lysozyme and cofilin addition, block and simple arrows, respectively. (c) Effect of cofilin on the bundling of 4 μM ANP-Mg-F-actin (squares) and cross-linked ANP-Mg-F-actin (diamonds). Bundling was monitored by low speed sedimentation as described in Materials and Methods. Error bars correspond to mean errors from three independent experiments.

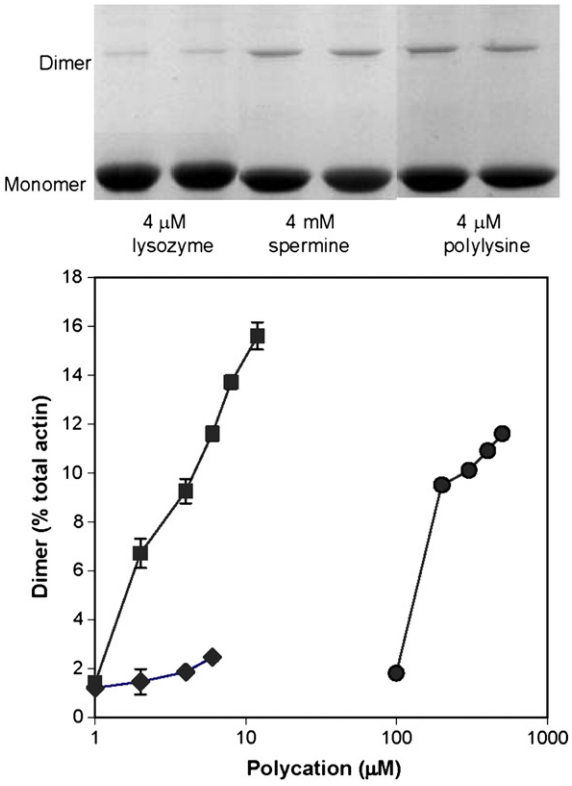


Fig. 6. Detection of antiparallel interfilament dimers in Mg-F-actin bundles by MTS-1 cross-linking. 4 μM Mg-F-actin – bundled by polycations – treated with 3 μM MTS-1 and analyzed by SDS-PAGE as described in Materials and methods. Squares, polylysine; diamonds, lysozyme; full circles, spermine. Error bars correspond to mean errors from three independent experiments.

that the bundling process starts during the nucleation of actin filaments, via the assembly of nuclei or the initial short filaments. The strong attraction between actin molecules and filaments due to electrostatic

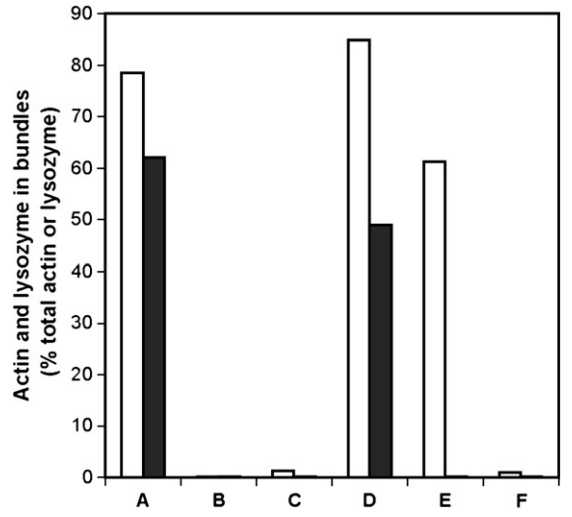


Fig. 7. Dissolving MTS-1 cross-linked and uncross-linked Mg-F-actin bundles by DTT and NaCl. 4 μM Mg-F-actin was bundled by 6 μM lysozyme at low ionic strength and cross-linked with 3 μM MTS-1 as described in Materials and methods. NaCl (100 mM) and DTT (1 mM) were added and the samples were centrifuged at 16 K for 8 min. The supernatants were analyzed for the actin and lysozyme content by SDS-PAGE. Additions to 4 μM Mg-F-actin and 6 μM lysozyme: (A), none; (B), 100 mM NaCl; (C), 100 mM NaCl and 1 mM DTT; (D), 3 μM MTS-1; (E), 100 mM NaCl and 3 μM MTS-1; (F), 3 μM MTS-1, 100 mM NaCl and 1 mM DTT and. Amount of actin in bundles as percentage of total actin, empty bars; amount of lysozyme in bundles as percentage of total lysozyme, full bars. Error bars correspond to mean errors from three independent experiments.

interactions with polycations is indicated also by the low critical concentration of polylysine bundled actin at low ionic strength conditions.

As reported earlier [4,15,17], the extent of actin filament bundling increases with polycation concentration both at low and physiological ionic strength. At physiological ionic strength about an order of magnitude higher polycation concentration is needed for filament bundling than at low ionic strength, as the monovalent cations (at relatively high concentrations) compete with polyvalent cations for the negatively charged actin and screen the effect of polycations. The bundling efficiency of polycations correlates with their net positive charge. However, the electrostatic interactions of positively charged proteins with actins may be enhanced by specific hydrophobic interactions, as is most likely the case for the calponin-actins system.

The very fast bundling of Mg-F-actin filaments upon the addition of polycations (at low ionic strength) suggests that these bundles must contain oppositely oriented actin filaments. The bundling is too fast to allow for the randomly oriented filaments to pre-orient by diffusion into parallel filaments. This is confirmed by our findings that Cys-374 of an actin protomer can be cross-linked to Cys-374 on another actin protomer in the bundle. According to the known structure of actin filaments [37], such cross-links by MTS-1 cannot occur between Cys-374 residues within individual filaments and must involve actins protomers from neighboring filament in the bundle. The proposed arrangement is similar to that in pPDM cross-linked actin paracrystals formed by 20–50 mM $MgCl_2$ [38].

The disassembly of bundles (Fig. 2c) is even faster than their formation (Fig. 2c) from actin filaments. The assembly, which is a bimolecular reaction, also involves movement and adjustment of filaments relative to each other, and thus follows biphasic kinetics. This does not happen during the disassembly, which is unimolecular. This is probably the reason for the disassembly being faster than the assembly of bundles.

The short span (5.4 Å) of the MTS-1 cross-linker of Cys-374 residues located on neighboring filaments in polycation induced actin bundles (Fig. 6 and Ref. [35]) indicates a tight packing of these filaments in the bundles. The degree of cross-linking increased with the polycation concentration and depended also on the nature of the polycation. A relatively high degree (~10–15%) of cross-linking was obtained in the actin-polylysine and actin-spermine systems, but less cross-linking (~2.5%) was detected in the actin-lysozyme bundles. The low level of cross-linking in the later case is presumably caused by the intercalation of lysozyme between the actin filaments [19,39], which increases the interfilament distance. Chemically cross-linked bundles, unlike the uncross-linked ones, do not disassemble in the presence of 100 mM NaCl (Fig. 7). This supports the assumption that these are interfilament cross-links, which connect neighboring and oppositely oriented filaments in the bundle.

Cofilin, an actin filament severing and depolymerization promoting protein [28,40,41] was found to disassemble lysozyme- and polylysine-induced bundles. Its effect is maximal at equimolar concentration of cofilin to actin. At physiological ionic strength, cofilin disassembles bundles fast and efficiently, especially the lysozyme-induced bundles. Unbundling by cofilin is based mostly on its severing action, since intrafilament cross-linking completely inhibits the cofilin-induced disassembly of bundles. One may speculate that the three net negative charges of yeast cofilin [42,43] can also facilitate disassembly. Cofilin's charges add to the net negative charge of actin protomers (over 50%) and, therefore, increase the polycation concentration required for the bundling of actin. The polysaccharide polyanion heparin, which is widely used for dissolving blood clots, also dissolves lysozyme-induced actin bundles by competing with the negatively charged actin for the polycation [36]. The unbundling effect of heparin and cofilin is cooperative since small amounts of heparin, which alone only slightly promote disassembly, facilitate strongly the unbundling by cofilin.

Bundles of actin filaments induced by two biologically relevant polycations, spermine and lysozyme, were studied in this work. Spermine bundles actin filaments only at millimolar concentration (4 mM) at

physiological ionic strength. However, the presence of relatively high concentration of spermine during sperm activation and neuron repair [44,45], which in part rely on actin bundles, raises the possibility that spermine contributes to bundle formation *in vivo*. The physiological or pathophysiological role of lysozyme is more likely, since both lysozyme and actin are present in high concentration in the pulmonary airways of cystic fibrosis patients [18,19]. Actin-lysozyme bundles are formed in the airways, which are stable at physiological ionic strength, and can contribute significantly to the accumulation of viscous sputum [21], which is the primary cause of long-term bacterial infections and respiratory failure observed in this ailment. Cofilin, which is present also in extracellular fluids [46], disassembles lysozyme-actin bundles and may have the potential to decrease the viscosity of the sputum and to alleviate the symptoms of cystic fibrosis. The unbundling of actin by cofilin is significantly increased by the heparin polyanion.

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