Introduction

Actin is the most abundant protein in the eukaryotic cell. It is a highly dynamic structural protein with multiple functions in cell physiology which include cytoskeleton formation, cell division, motility, adhesion, signaling and more [1]. Actin exists in either monomer-globular (G) or polymer-filament (F) form. These forms can interconvert into each other by a plethora of different factors. G-actin, which is negatively charged, is polymerized into F-actin, which is positively charged, by MgCl2 or NaCl. LL-37 bundles Mg-actin filaments both at low and physiological ionic strength when in equimolar or higher concentrations than those of actin. The LL-37-induced actin bundles are significantly less sensitive to increase in ionic strength than those induced by scrambled LL-37 and lysozyme. LL-37 in concentrations lower than those needed for actin polymerization or bundling, accelerates cleavage of both monomer and polymer actin by subtilisin. Our results indicate that the LL-37-actin interaction is partially electrostatic and partially hydrophobic and that a specific actin binding sequence in the peptide is responsible for the hydrophobic interaction. LL-37-induced bundles, which may contribute to the accumulation of sputum in cystic fibrosis, are dissociated very efficiently by DNase-1 and also by cofilin.

Abstract

Actin exists as a monomer (G-actin) which can be polymerized to filaments (F-actin) that under the influence of actin-binding proteins and polycations bundle and contribute to the formation of the cytoskeleton. Bundled actin from lysed cells increases the viscosity of sputum in lungs of cystic fibrosis patients. The human host defense peptide LL-37 was previously shown to induce actin bundling and was thus hypothesized to contribute to the pathogenicity of this disease. In this work, interactions between actin and the cationic LL-37 were studied by optical, proteolytic and surface plasmon resonance methods and compared to those obtained with scrambled LL-37 and with the cationic protein lysozyme. We show that LL-37 binds strongly to CaATP-G-actin while scrambled LL-37 does not. While LL-37, at superstoichiometric LL-37/actin concentrations polymerizes MgATP-G-actin, at lower non-polymerizing concentrations LL-37 inhibits actin polymerization by MgCl2 or NaCl. LL-37 bundles Mg-F-actin filaments both at low and physiological ionic strength when in equimolar or higher concentrations than those of actin. The LL-37-induced bundles are significantly less sensitive to increase in ionic strength than those induced by scrambled LL-37 and lysozyme. LL-37 in concentrations lower than those needed for actin polymerization or bundling, accelerates cleavage of both monomer and polymer actin by subtilisin. Our results indicate that the LL-37-actin interaction is partially electrostatic and partially hydrophobic and that a specific actin binding sequence in the peptide is responsible for the hydrophobic interaction. LL-37-induced bundles, which may contribute to the accumulation of sputum in cystic fibrosis, are dissociated very efficiently by DNase-1 and also by cofilin.

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* E-mail: gladlab@ekmd.huji.ac.il

These authors contributed equally to this work.
of LL-37 attached to actin bundles was reported to be significantly reduced [6]. Geholin and polyanions dissolve actin bundles [30] and restore LL-37’s antibacterial activity [6]. Despite these significant advances, important processes, such as polymerization of G-actin by LL-37, kinetics of bundle formation, characterization of actin-LL-37 interaction at molecular level and the reaction of LL-37-induced bundles with actin binding and severing proteins remain uncharacterized and poorly understood.

In this study, we examined the LL-37-induced polymerization of MgATP-G-actin and the bundling of Mg-F-actin, and compared them to the well characterized polymerization and bundling of actin by the antibacterial polycationic protein lysozyme [20]. We show that LL-37 polymerizes actin only at concentrations greater than twice that of actin. Low concentrations of LL-37, which are not sufficient to polymerize actin, inhibit actin polymerization induced by MgCl₂ or NaCl. LL-37-induced F-actin bundles are less sensitive to ionic strength when compared to sLL-37 (scrambled LL-37) or lysozyme induced bundles. Substoichiometric LL-37 concentrations relative to actin accelerate the subtilisin digestion of the protein. Our results indicate that in addition to electrostatic interactions there are specific hydrophobic interactions between LL-37 and actin. These interactions may involve the DNase I binding (D) loop since DNase I dissociates LL-37 induced F-actin bundles very efficiently.

Materials and Methods

Materials

N-(1-pyrene) maleimide was obtained from Molecular Probes (Eugene, OR). Hen lysozyme, DNaseI, ATP, ADP, dithiotreitol (DTT), and EGTA were purchased from Sigma Chemical Co. (St Louis, MO). LL-37 (LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES) peptide and scrambled LL-37 (sLL-37) peptide (GKLKRFESKKIGEFLKTEFRVFIRDKLDN-RISVQR) were purchased from (Genemed Synthesis Inc., San Antonio, TX). The peptides were purified by HPLC and purity (greater than 90%) was determined by Mass Spectrometry. Yeast cofillin was a generous gift from Prof. Emil Reisler Dept. of Chemistry and Biochemistry, Univ. of California Los Angeles CA.

Preparation of Actin

CaATP-G-actin was prepared from acetone dried powder derived from the back and leg muscles of rabbit by the method of Spudich and Watt [31] that even without gel filtration yields highly homogeneous actin in purity greater than 90%. CaATP-G-actin was stored in a buffer containing 5 mM TrisHCl, 0.2 mM CaCl₂, 0.2 mM ATP, 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 MgCl₂ at room temperature for 5 min. MgATP-G-actin was diluted for further treatments in MgATP-G-buffer containing 5 mM MOPS, 0.1 mM MgCl₂, 0.2 mM EGTA, 0.2 mM ATP and 0.5 mM DTT, pH 7.4. Mg-F-actin was polymerized from MgATP-G-actin or CaATP-G-actin by 30 min incubation with 2 mM MgCl₂ at room temperature. The concentration of un-labeled rabbit skeletal muscle γ-actin was determined spectrophotometrically using the extinction coefficients E²₉₀ = 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 290 nm to 290 nm). Molecular masses of skeletal actin, yeast cofillin, hen-lysozyme and LL-37 were assumed to be 42 kDa, 15.9 kDa, 14.3 kDa and 4.5 kDa respectively.

Pyrene Labeling

Labeling of Mg-F-actin at Cys-374 with pyrene maleimide was carried out according to Kouyama and Mihashi [32] with some modifications. CaATP-G-actin was filtered through a PD-10 column (GE Healthcare) equilibrated with β-mercaptoethanol-free CaATP-G-buffer. After filtration, actin (1 mg/ml) was polymerized by 2 mM MgCl₂ and 100 mM KCl at room temperature for 30 min, and reacted with pyrene maleimide (16 μg/ml) on ice, for one hour. The reaction was terminated with 1 mM DTT. Labeled F-actin was centrifuged at 129,151 × g for two hours, the pellet was resuspended in Ca-ATP-G-buffer and depolymerized by dialyzing in this buffer for over 36 hours at 4°C. Finally, actin was centrifuged again at 129,151 × g for two hours. The supernatant contained the purified pyrene-labeled CaATP-G-actin. The concentration of modified actin was determined by the procedure of Bradford [33] using unmodified actin as a standard. The extent of labeling, which was measured by using pyrene extinction coefficient E₃₄₄nm = 22000 cm⁻¹M⁻¹, was ~100%.

Fluorescence and Light Scattering Measurements

Actin polymerization was followed as an increase in fluorescence of pyrene-labeled G-actin [32], which was added to unlabeled G-actin in 10–15%. 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 spectrophotometer (Photon Technology Industries, South Brunswick, NJ). Bundling of Mg-F-actin was followed by increase in light scattering [20]. The time course of light scattering changes was also measured in a PTI spectrophotometer, with both excitation and emission wavelengths adjusted to 450 nm. All fluorescence and light scattering measurements were carried out at 22°C.

Monitoring Bundling by Low Speed Sedimentation

After addition of polycations, actin samples were centrifuged at 20,800 × g at 4°C for 8 min (in an Eppendorf centrifuge). The supernatants were run using SDS-PAGE and analyzed by densitometry using the TINA 2.07d software.

Binding Measurement of LL-37 or Scrambled LL-37 to G-actin by Surface Plasmon Resonance

The preparation of the sensor surfaces and the interpretation of the sensorgrams were performed using BIACORE 3000 system (GE). For the immobilization procedure, a CM5 chip (GE) was activated using amine coupling kit (GE) by the standard protocol. The running buffer used was 10 mM HEPES buffer pH 7.4, 150 mM NaCl, and 0.005% tween 20. The flow rate during immobilization was 5 μl/min. Following activation, actin (10 μg/ ml) was injected to give a final level of actin binding of 1500–1800 RU (Resonance Units). The remaining activated groups were blocked by injection of 1 M ethanolamine, pH 8.5. All experiments were conducted at 25°C using a flow rate of 40 μl/min. Series of LL-37 concentrations (up to 500 nM) were injected. Each injection was followed by regeneration using 10 μl of 10 mM NaOH. Evaluation was done using BIAevaluation software (version 4.1). Affinity constant was calculated by using the 1:1 Langmuir model. For sLL-37 a steady state affinity model was used due to fast equilibration. Binding was measured as Resonance Units (RU)/s. The SPR signal obtained in each individual reaction cycle was recorded as a sensogram, which is a real-time pattern plotted in RU versus time [8]. The KD. Experiments were repeated three times.
Results

Polymerization of MgATP-G-actin by LL-37

In low ionic strength MgATP-G-buffer, LL-37 was found to polymerize MgATP-G-actin (Figure 1A). The onset of polymerization was fast and its extent was dependent on the concentration of LL-37. Significant polymerization was observed when the concentration of LL-37 was three times higher than that of MgATP-G-actin (6 µM LL-37, and 2 µM actin) (Figure 1A). Lysozyme polymerized MgATP-G-actin at half of the concentration of MgATP-G-actin (1 µM lysozyme and 2 µM actin) (Figure 1B). One may suppose that lysozyme polymerizes G-actin at lower concentrations than LL-37; because its net positive charge is higher than that of LL-37 [6]. However, LL-37’s affinity to MgATP-G-actin also seems to be high as implied by the finding that LL-37 affects G-actin even when its concentration is too low to induce polymerization. Under these conditions, LL-37 inhibits polymerization induced by 2 mM MgCl₂ or 100 mM NaCl (Figure 2A) while similar concentrations of lysozyme have no inhibitory effect (Figure 2B). These results may indicate that monomeric actin forms complexes with LL-37 that at low LL-37 concentrations inhibit salt-induced actin polymerization.

Bundling of MgF-actin by LL-37

Bundling kinetics of MgF-actin by LL-37, lysozyme and scrambled LL-37 were studied by increase in light scattering and the extent of bundling was determined using low speed centrifugation. The bundling of MgF-actin by LL-37 is also shown by electron microscopy (Figure S1). MgF-actin bundle formation was very fast both with LL-37 (Figure 3A) and lysozyme [20]. The extent of bundling was dependent on the concentration and the nature of the polycation used (Figure 3B). At low ionic strength more than 50% of MgF-actin was bundled upon addition of lysozyme (1 µM) to MgF-actin (4 µM). A similar degree of bundling was observed with LL-37 when its concentration was equal to that of actin (4 µM) (Figure 3B). At low ionic strength scrambled LL-37 induced significant bundling when its concentration was 2.5 fold higher than that of MgF-actin (Figure 3C). These results are similar to those obtained for MgATP-G-actin polymerization by lysozyme and LL-37. Bundling of F-actin by polycations is ionic-strength-sensitive and bundles induced by polycations dissociate (unbundle) at physiological ionic strength [20]. This is also observed with lysozyme where a six fold higher concentration of lysozyme (6 µM) was needed for half maximal bundling of 4 µM Mg-F-actin at physiological (100 mM NaCl) than at low ionic strength (1 µM lysozyme) (Figure 3B). With scrambled LL-37, whose net number of positive charges are identical to those of LL-37, even 14 µM were insufficient to cause significant bundling in the presence of 100 mM NaCl, while at low ionic strength more than 60 percent of Mg-F-actin bundled at 10 µM concentration of sLL-37 (Figure 3C). The results show the competing effect of ionic strength and polycation concentration on bundling. Unlike with lysozyme and sLL-37, the kinetics (Figure 3A) and the extent (Figure 3B and C) of bundling by LL-37 are practically identical at low and at physiological ionic strength. The decreased salt sensitivity of LL-37-induced bundles may indicate that the interactions between LL-37 and actin are partially electrostatic and partially hydrophobic.

Effect of LL-37 on Subtilisin Digestion of CaATP-G, MgATP-G-actin and Mg-F-actin

Subtilisin at low concentrations cleaves G-actin between Met-47 and Gly-48 [35]. In order to cleave F-actin, a magnitude higher concentration of subtilisin is needed. The subtilisin digestion of actin is dependent on the structure of the protein.
For example, the exchange of tightly bound ATP to ADP in the nucleotide binding cleft, significantly decreases the rate of actin cleavage by subtilisin [36]. Addition of cofilin affects MgActin’s structure, and strongly accelerates the digestion of actin filaments by subtilisin [37]. In light of our assumption that the binding of LL-37 to actin is partially hydrophobic, and may affect actin structure, we examined the effect of LL-37 on actin digestion by subtilisin (Figure 5). CaATP- and MgATP-G-actin (8 μM) were digested by subtilisin (4 μg/ml) for 2 minutes. Mg-F-actin (8 μM) was digested in the presence and absence of 200 mM NaCl, by 20 μg/ml subtilisin for 30 min. Digestions were carried out in the presence or absence of 6 μM LL-37, which in all cases enhanced the subtilisin cleavage in a statistically significant manner (Figure 5). These results indicate that LL-37 binds both the monomer and polymer forms of actin. It also binds F-actin in the presence of 200 mM NaCl, which abolishes electrostatic interactions between F-actin and LL-37 (Figure 4). Thus, the binding of LL-37 to actin is at least partially hydrophobic and induces changes in actin structure, which is manifested in increased sensitivity toward subtilisin.

Surface Plasmon Resonance Measurements of Binding of LL-37 to Immobilized G-actin

In light of the subtilisin digestion results, which showed that LL-37 binds G-actin and affects its structure, we measured the dissociation constants of LL-37 and of scrambled LL-37 from CaATP-G-actin using the SPR BIACORE approach (Figure 6). CaATP-G-actin was bound to the sensor chip at low ionic strength and washed. LL-37 or sLL-37 were applied to the surface-bound G-actin followed by washing with the 150 mM NaCl supplemented CaATP-G-buffer (G-actin was not polymerized by this high ionic strength buffer, because it was bound to the chip surface). The results presented in Figure 6A show that LL-37 binds strongly to G-actin (Table 1) in the presence of 150 mM NaCl, which masks most of the electrostatic interactions between actin and LL-37. The KD value of LL-37 binding obtained in this measurement is slightly different when compared with the results of the bundling experiments (Figure 3C). This might result from the fact that the binding of LL-37 was measured to monomeric CaATP-G-actin, while the bundling experiments were carried out with polymeric MgF-actin. sLL-37 binds very weakly to G-actin under the same conditions (Figure 6B, Table 1). These results indicate a strong, sequence dependent hydrophobic interaction between LL-37 and actin. It appears that LL-37 contains an actin binding sequence, which is perturbed by scrambling, that abolished the hydrophobic interaction.

Dissociation of LL-37-induced Mg-F-actin Bundles by DNase1 and Coflin

Bundles of actin filaments are major contributors to the viscosity of sputum in the airways of cystic fibrosis patients. LL-37, which also has been found in the sputum, may contribute to actin bundle formation [38]. Gelsolin, an actin filament severing protein was found to significantly reduce the viscosity of sputum [30] and dissociate LL-37-induced actin filament bundles [38]. Here, we studied the effect of coflin [39] and DNase1 on the stability of LL-37-induced bundles.

Coflin, like gelsolin, is an actin-filament severing protein [39]. DNase1, depolymerizes F-actin and forms a tight complex with the actin monomer by binding to its DNase1 binding loop (D-loop) [40]. The effect of 5 μM DNase1 (Figure 7A) or coflin (Figure 7B) on 4 μM Mg-F-actin bundled with 4–9 μM LL-37 in the presence or absence of 100 mM NaCl was examined. Both DNase1 and coflin were found to dissociate actin bundles and their effect decreased with the increase in the LL-37 concentration. This indicates a competition for a binding site on actin between LL-37 and DNase1 or coflin. Dissociation was more efficient in the presence of 100 mM NaCl than in low ionic strength, indicating that the increased ionic strength contributed to the disassembly of the bundles. DNase1 dissociated F-actin bundles far more efficiently than coflin. This was especially conspicuous at low ionic strength, where coflin had a poor dissociating effect and almost did not affect bundling at high (9 μM) LL-37 concentrations.

The kinetics of dissociation of LL-37-induced Mg-F-actin bundles was also studied (Figure 8). In this experiment, LL-37 (9 μM) was added to MgF-actin (4 μM), which induced immediate bundling. This was followed by the addition of
6 μM DNase1, cofilin, or 200 mM NaCl. Bundling and dissociation was monitored as a change in light scattering. Dissociation was complete and very fast with 200 mM NaCl, fast but only partial with 6 μM cofilin and relatively slow but led to near complete unbundling with 6 μM DNase1. The very fast dissociation by NaCl may indicate the abolishment of the interactions between the filaments. The dissociation by cofilin may be caused by severing while the slow scattering decrease induced by DNase1 can be due to depolymerization of actin filaments. These assumptions were further examined by investigating the mechanism of dissociation of LL-37-induced Mg-F-actin bundles by DNase1 and cofilin using low and high speed sedimentation (Figure 9). G-actin, which does not sediment even by high speed centrifugation, was separated from F-actin, which was sedimented by one hour centrifugation at 352,271 g. The bundled F-actin, unlike the unbundled one, is sedimented at low speed centrifugation (20,800 g, 8 min). Thus, the three actin forms (G-, unbundled F- and bundled F-actin) were separated from each other by low and high speed centrifugations. Addition of 6 μM DNase1 and 9 μM cofilin to

Figure 3. LL-37, scrambled LL-37 and lysozyme induced bundle formation of Mg-F-actin at low and physiological (100 mM NaCl) ionic strength. (A) Kinetics of LL-37-induced bundle formation: LL-37 (2–8 μM) was added to Mg-F-actin (2 μM) and bundle formation was followed as an increase in light scattering at 350 nm. Presented data are representative of three independent experiments. (B) Extent of LL-37 and lysozyme induced bundle formation: LL-37 or lysozyme (0.5–9 μM) were added to Mg-F-actin (4 μM). Samples were centrifuged at 20,800 g, for 8 min and the supernatants were analyzed by SDS-PAGE and densitometry. (C) Extent of LL-37 and scrambled LL-37 induced bundle formation: LL-37 or scrambled LL-37 (2–14 μM) were added to Mg-F-actin (4 μM) and bundle formation was measured as in (B). The presented data are mean and standard deviation of three independent experiments in (B) and (C).

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4 μM Mg-F-actin (bundled by 9 μM LL-37) induced bundle disappearance at 100% and 73% respectively (Figure 9). However, as indicated by the high speed centrifugation, the cofilin treated F-actin bundles remained polymerized, while the DNase1 treated one completely depolymerized (as shown by the lack of sedimentation). These results are in accordance with the well known depolymerizing effect of DNase1 through the formation of G-actin-DNase1 complex [40] and cofilin severing [41] of the actin filament. Finally, we compared the dissociation of LL-37- (Figure 10A) or lysozyme-induced (Figure 10B) actin bundles by DNase1 and cofilin. In this experiment MgF-actin (4 μM) was bundled by 9 μM LL-37 or lysozyme in the presence of 100 mM NaCl. DNase1 or cofilin were subsequently added in increasing concentrations to the bundled actin, which after 10 min incubation was separated from the unbundled actin by low speed sedimentation. The dissociation of LL-37-induced actin bundles was found to be more extensive with DNase1 than with cofilin and the unbundling effect of the two proteins was additive (Figure 10A). Contrary to the LL-37-induced bundles, the dissociation of the lysozyme-induced bundles is more extensive with cofilin than with DNase1. These findings indicate that there are structural differences between the two types of bundles. It seems that the LL-37-induced bundles are more easily depolymerized by DNase1 while for the lysozyme-induced ones the filament severing is the preferred unbundling process.
LL-37 is a polycationic 37-mer human host defense peptide with multiple functions that include antimicrobial, immunoregulatory and tissue repair activities [21, 42]. It polymerizes G-actin and bundles F-actin if its concentration is higher than that of actin and reacts with actin monomers or protomers also at low concentrations, which do not polymerize actin. The LL-37-induced bundles could play a role in cellular actin dynamics and may participate in the formation of cytoskeletal structures. LL-37 has been shown to have many cellular functions, including chemotaxis, cytokine release, tissue regeneration, inhibition of immunostimulation and apoptosis [21, 43]. It is assumed that the direct or indirect effects of LL-37 on actin dynamics have a significant role on antibacterial defense mechanism of lung epithelial cells [27].

LL-37 polymerizes G-actin at concentrations greater than twice than that of actin. At 4 μM LL-37, a concentration not sufficient to cause polymerization (Figure 1A), LL-37 still reacts with actin monomers, as shown by the LL-37 inhibition of MgCl2- and NaCl-induced actin polymerization (Figure 2A). This finding may indicate the existence of a stable LL-37-actin- monomer complex. This assumption is further supported by the acceleration of the subtilisin digestion of both CaATP-G-actin, MgATP-G-actin and...
MgF-actin by substoichiometric LL-37 concentrations relative to actin. Subtilisin digestion of actin is highly sensitive to subtle changes in actin structure [37]; therefore, the increased subtilisin sensitivity of actin in the presence of LL-37 indicates LL-37-induced changes in actin’s conformation. LL-37 enhanced subtilisin digestion of Mg-F-actin also in the presence of 200 mM NaCl, which dissolves LL-37-induced bundles by masking the electrostatic interactions between F-actin and LL-37. The observation that LL-37 affects actin structure even in the absence of electrostatic interactions indicates the existence of specific hydrophobic interactions between the protein and the peptide. The present investigation, which showed that F-actin bundles induced by LL-37 are significantly more resistant to increasing ionic strength compared to those formed by lysozyme and other polycations (Figure 4 and [38]), supports the existence of hydrophobic interactions between F-actin and LL-37. This was further confirmed by measuring the binding of LL-37 and scrambled LL-37 to actin by surface plasmon resonance, which showed that LL-37 strongly binds G-actin, while sLL-37 does not (Figure 6). This indicates the existence of a special actin binding sequence in LL-37, which is responsible for the hydrophobic interactions between the peptide and actin. These interactions help to stabilize the bundles formed by the polycationic peptide via neutralization of the repulsive negative charges of actin filaments.

DNase1, a G-actin binding and F-actin depolymerizing protein [40] and cofilin, an actin filament severing and depolymerization promoting protein [41], [44] and [45] were shown in the present study to disassemble LL-37- and lysozyme-induced actin bundles. DNase1 unbundles polycation-induced bundles by depolymerizing actin filaments attached to each other. This is indicated by the finding that essentially no F-actin remained in the solution after DNase1 caused disassembly of the bundles. Cofilin dissociates LL-37-induced bundles by severing filaments, as actin remained polymerized after cofilin addition (Figure 10). The DNase1-caused dissociation of LL-37-induced bundles is slower but much more efficient than the disassembly induced by cofilin. On the other hand, cofilin is more efficient than DNase1 at dissociating lysozyme-induced actin filament bundles. The strong dissociating effect of DNase1 on LL-37-induced bundles may indicate that DNase1 and LL-37 compete for the same site of the actin protein, specifically for the DNase1 binding (D) loop.

In cystic fibrosis, which is the most common fatal inherited disease in the western world [29], large quantities of F-actin and DNA released from lysed inflammatory cells are found in the surface airway liquid, together with polycationic antimicrobial polypeptides, such as LL-37, lysozyme, β-defensin and lactoferrin [38]. These polycations promote the formation of actin and DNA bundles by neutralizing the repulsive negative charges of the F-
actin and DNA polyanions [18], [20]. The bundles have a significant role in symptom aggravation in cystic fibrosis patients. LL-37 was previously shown to bundle actin in the sputum of cystic fibrosis patients [6]. However, in sputum of cystic fibrosis patients LL-37 concentration was found to reach 15 mg/ml (3 mM) [46] while extracellular F-actin ranged between 0.1–5 mg/ml (2–120 mM) [30]. This LL-37/actin ratio is seemingly lower than the superstoichiometric one found here necessary for actin bundling. It is possible that in inflamed site, temporary local LL-37 concentrations together with those of other cationic actin bundling proteins (such as lysozyme) reach sufficient concentrations to induce bundling of actin released from lysed cells.

LL-37-induced F-actin bundles have a special impact on the disease because they form at relatively low LL-37 concentrations and are stable at physiological ionic strength, unlike those induced by low micromolar concentrations of lysozyme, which are dissociated at physiological ionic strength. Another important characteristic of LL-37-induced bundles is their very efficient DNase1 induced disassembly. DNase1 dissociates DNA bundles by cleaving the DNA double helix. This activity is inhibited by actin, which is tightly bound to DNase1. Therefore, a specially constructed human recombinant DNase1, whose actin binding site is missing was developed to dissociate DNA bundles in the airways of cystic fibrosis patients [47]; however, this DNase1 is not suitable to disassemble actin bundles due to the fact that it does not react with actin. In light of the efficient dissociation of LL-37-induced actin bundles by native, non-recombinant DNase1 (used in this study), it would be worth to study the use of a mixture of non-recombinant and recombinant DNase1, the former to dissociate actin and the latter to sever DNA bundles, for cystic fibrosis treatment.

Supporting Information

Figure S1 LL-37 induces bundling of Mg-F-actin. Scanning electron microscopy showing 2 mM Mg-F-actin before (A) and after (B) incubation with 4 mM LL-37.

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Author Contributions

Conceived and designed the experiments: AM GB AS EB. Performed the experiments: AM AS, EB. Analyzed the data: AM GB AS EB. Contributed reagents/materials/analysis tools: AM GB AS EB. Wrote the paper: AM GB EB.

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Figure 10. Comparison of DNase1 and coflin dissociation of LL-37- and lysozyme-induced Mg-F-actin bundles. Mg-F-actin (4 mM) was bundled by 9 mM LL-37 or lysozyme in the presence of 100 mM NaCl. After 10 min of incubation, DNase1 or coflin were added in increasing concentrations to bundled actin. After 30 min of incubation, the samples were centrifuged at 20,800 x g for 8 min and the supernatants were analyzed by SDS-PAGE and densitometry. (A) Unbundling of LL-37-induced Mg-F-actin bundles. (B) Unbundling of lysozyme-induced Mg-F-actin bundles. The presented data are mean and standard deviation of three independent experiments.

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