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Interaction of the core fragments of the LL-37 host defense peptide with actin

Asaf Sol,†[a] Guangshun Wang,†[b] Edna Blotnick,[c] Radha Golla,[b] Gilad Bachrach†*[a] and Andras Muhlrad†[a]

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Host defense peptides are effector molecules of the innate immunity that possess antimicrobial and health-promoting properties. Due to their potential therapeutic activities, host defense peptides are being explored as alternatives for antibiotics. The human LL-37 and its shorter, cost-effective, bactericidal core peptide derivates have been suggested for their therapeutic potential. Bacteria evade host defense peptides by proteolytic inactivation. Actin released from necrotized cells and abundant in infected sites was shown to bind and protect LL-37 from microbial proteolytic degradation, and to enable the peptide's antimicrobial action despite the presence of the proteases. Here, we characterized the interactions of the 10–13 residues long LL-37 core peptides with actin. We show that the LL-37 core peptides associate with actin with a lower affinity than that of LL-37. Their association with actin, which is very ionic strength sensitive, is mainly based on electrostatic interactions. Likewise, the antimicrobial activity against *Escherichia coli* of the minimal antimicrobial peptide KR-12 but not FK-13 nor LL-37 is also very sensitive to salts. In addition, the antimicrobial activity of the FK-13 core peptide is protected by actin against the tested bacterial proteases in a similar manner to that of LL-37, supporting its potential for therapeutic use.

1. Introduction

Host defense peptides are components of the innate immunity that mediate a broad range of antimicrobial activity together with immunoregulatory and tissue repair functions. Though highly diverse, antimicrobial peptides usually share the features of a net positive charge and the ability to adopt an amphipathic structure in solution. LL-37 is the only human member of the cathelicidin family of host defense peptides. It has multiple activities, including antimicrobial,[1] wound healing,[4] chemoattraction,[6] tissue regeneration,[20] inhibition of immunostimulation[14] and apoptosis.[12] LL-37 consists of 37 amino acids and start with a pair of leucines (Table 1). Of this sequence, a 13 amino acid fragment starting with FK (named FK-13) possesses a number of the LL-37's antimicrobial and anticancer activities.[10,23] It was found that an even smaller fragment without the N-terminal phenylalanine residue, called KR-12, retains the antimicrobial activity of the molecule.[10] However, further removal of residues either from the C- or the N-terminus of KR-12 leads to the loss of antimicrobial activity.[21] Therefore, KR-12 is considered the smallest core antimicrobial fragment of LL-37 (Table 1).

Periodontitis is a chronic inflammatory disease that leads to destruction of the attachment apparatus of the teeth. Deficiency of salivary LL-37 in patients with morbus Kostmann[13] or with Papillon–Lefevre syndrome[5] was previously correlated with the overgrowth of *Aggregatibacter actinomycetemcomitans* and the appearance of severe periodontitis. *Porphyromonas gingivalis* is an oral anaerobe and the pathogen most associated with chronic periodontal disease.[7,8,16] This oral pathogen expresses and secretes the Arg-gingipains and Lys-gingipain cysteine proteases that cleave after arginine and lysine residues, respectively. Being positively charged, arginine and lysine are highly represented in host defense peptides, including LL-37 (which has five arginines and six lysines), making gingipains a good model for studying the microbial proteolytic evasion of host defense peptides.

Actin, the most abundant structural protein in the eukaryotic cell exists as a monomer (globular, G-actin) or a polymer (actin filament, F-actin). These actin species transform into one another by different factors. G-actin is polymerized into F-actin by an increase in monovalent or divalent cation concentration and by specific positively charged proteins and peptides.[15] F-actin filaments may form bundles *via* actin bundling proteins.

As mentioned above, LL-37 is a polycationic polypeptide containing 6 net positive charges. It interacts with the negatively charged actin. LL-37 reacts with both the monomer-globular (G) and polymer-filament (F) forms of actin[17,18,24] When LL-37 is

[a]*Institute of Dental Sciences, The Hebrew University-Hadassah School of Dental Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel. E-mail: giladba@ekmd.huji.ac.il; Fax: +972 2675 8561; Tel: +972 2675 7117*

[b]*Department of Pathology and Microbiology, University of Nebraska Medical Center, Nebraska Medical Center, Omaha, USA*

[c]*Department of Medical Neurobiology, The Institute for Medical Research-Israel-Canada, The Hebrew University of Jerusalem, Jerusalem, Israel*

† These authors contributed equally.

Table 1 Peptides used in this study[a]

Peptide	Sequence	Net charge	# of hydrophobic residues	Boman index (kcal mol^{-1})	*K*-dissociation with actin M(−1)	MIC[b] (μM)
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	+6	13	2.99	1.86×10^{-7}	1.1
FK-13	FKRIVQRIKDFLR	+5	6	3.48	1.86×10^{-5}	3.3
KR-12	KRIVQRIKDFLR	+5	5	4.02	1.18×10^{-5}	6.6
KR-10	KRIVQRIKDF	+4	4	3.83	2.84×10^{-5}	[c]NI

[a] Calculated in the antimicrobial peptide database using the peptide calculator at http://aps.unmc.edu/AP/prediction/prediction_main.php.[22] Note that the C-termini of the core peptides are amidated. [b] Measured with *B. subtilis*. [c] No inhibition at concentrations ≤ 11 μM.

bound to F-actin, it is protected from proteolysis by the bacterial pathogen *Pseudomonas aeruginosa* and the highly proteolytic periodontal pathogen *Porphyromonas gingivalis*. Thus actin enables the antimicrobial activity of LL-37 in the presence of microbial proteases.[18] This actin-mediated protection of LL-37 may have physiological–immunological significance. LL-37 polymerizes G-actin to F-actin,[17] bundles F-actin[17,18,24] and can be cross-linked to both G- and F-actin by transglutaminase.[18] The binding of LL-37 to actin is based on both electrostatic and hydrophobic interactions. The latter is indicated by the relatively low ionic strength sensitivity of the actin bundling activity of LL-37 (ref. 17 and 18) in comparison to the ionic strength sensitivity of actin bundling by other polycations, such as lysozyme.[11] LL-37 binds to the His40–Lys50 segment of the DNase-I binding loop (D-loop) of actin according to evidence obtained using proteolytic and mass spectroscopy analyses.[18] The integrity of the actin's D-loop sequence is a prerequisite to the proper LL-37–actin interaction. Changes in the LL-37 sequence, such as scrambling, substitution of Gln22 to Ala, or replacement of the hydrophobic Ile20 and Ile24 residues with Ser, considerably weakens the LL-37–actin interactions, especially the hydrophobic interactions as shown by the dramatic decrease in binding of modified LL-37 to actin with the increase of NaCl concentration.[18]

The LL-37 derived antimicrobial core peptides, FK-13 and KR-12 were suggested as potential therapeutic agents.[21] Protection of these compounds by actin, from degradation–inactivation by microbial proteases might be essential for effective treatment with these antimicrobial peptides. Therefore, in this study we examined the interaction of the FK-13, KR-12, and KR-10 peptides with actin and compared the actin interaction and antimicrobial activity of these LL-37 fragments with that of the parent molecule.

2. Material and methods

2.1. Materials

ATP, ADP, and dithiotreitol (DTT) were purchased from Sigma Chemicals Co. (St Louis, MO). The sequences of the peptides used in this study are given in Table 1. LL-37 (LLGDFFRKSKE KIGKEFKRIVQRIKDFLRNLVPRTES), tetramethylrhodamine labeled LL-37 (f-LL-37), FK-13 (FKRIVQRIKDFLR), KR-12 (KRIVQRIKDFLR) and KR-10 (KRIVQRIKDF) were purchased from Genemed Synthesis Inc., (San Antonio, TX). The peptides were purified by HPLC (greater than 90% purity). Bacterial transglutaminase (TGase) was a generous gift of Prof. György Hegyi, Eötvös Loránd University, Budapest, Hungary.

2.2. Bacterial strains and growth conditions

Bacillus subtilis PY79 (a kind gift of Prof. S. Ben-Yehuda, The Hebrew University, Jerusalem) was grown in LB Broth (Difco, MD, USA) at 37 °C under aerobic conditions. *Porphyromonas gingivalis* ATCC 33277 (from our laboratory stock) was grown in Wilkins Chalgren Medium II, (Oxoid, UK) in anaerobic jars (Oxoid, UK) at 37 °C. For supernatant collection, 4 day cultures of *P. gingivalis* were centrifuged at 20 000 × *g* for 10 min and the supernatant was collected and transferred through a 0.2 μm filter (BD, USA). Bacterial purity was determined by phase contrast microscopy.

2.3. Protection assay

LL-37 or FK-13 (1.1 μM and 3.3 μM respectively) were incubated with or without 3 μM or 9 μM of F-actin for 10 min at 37 °C. 5 μl of protease-containing supernatant of *P. gingivalis* diluted 1/10 were added (or not) to the 50 μl reaction mixture, and incubated for 30 min at 37 °C. The reaction mixtures were added to wells of 96-well plates (Nunc, Denmark) containing 150 μL of *B. subtilis* PY79 cells (at mid-logarithmic growth) diluted 1 : 5000 in LB (to complete a 200 μl final volume). To measure the protection of the bacterial growth inhibition activity of LL-37, the plates were transferred to a GENIOS Microplate Reader (TECAN, Austria) and optical densities at OD 595 nm were measured during incubation at 37 °C every 17 minutes (after automated mixing/aeration for 500 seconds) to generate growth curves. The percent growth inhibition of each treatment was compared to the growth at the late logarithmic growth phase of untreated bacteria (0% growth inhibition).

2.4. Preparation of actin

CaATP-G-actin was prepared from acetone dried powder derived from the back and leg muscles of rabbits by the method of Spudich and Watt[19] without gel filtration which yields highly homogeneous actin in purity greater than 90%. CaATP-G-actin was stored in a buffer containing 5 mM TrisHCl, 0.2 mM $CaCl_2$, 0.2 mM ATP, 0.5 mM β-mercaptoethanol, pH 8.0 (CaATP-G-buffer). MgF-actin was polymerized from CaATP-G-actin by 30 min incubation with 2 mM $MgCl_2$ at room temperature. The

concentration of unlabeled rabbit skeletal muscle α-G-actin was determined spectrophotometrically using the extinction coefficients $E_{290}^{1\%} = 11.5\ cm^{-1}$ (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). The molecular masses of skeletal actin, LL-37, FK-13, KR-12 and KR-10 were assumed to be 42 kDa, 4.5 kDa, 1.74 kDa, 1.57 kDa and 1.27 kDa respectively.

2.5. Pyrene labeling of actin

The labeling of Mg-F-actin at Cys-374 with pyrene maleimide was carried out according to Kouyama and Mihashi[9] 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^{-1}) was polymerized by 2 mM $MgCl_2$ and 100 mM KCl at room temperature for 30 min, and reacted with pyrene maleimide (16 μg ml^{-1}) 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 the modified actin was determined by the procedure of Bradford[3] using unmodified actin as a standard. The extent of labeling, which was measured by using the pyrene extinction coefficient $E_{344\ nm} = 22\ 000\ cm^{-1}\ M^{-1}$, was ~100%.

2.6. Actin bundling

Following the addition of LL-37, FK-13, KR-12 and KR-10 to MgF-actin, the samples were centrifuged at 20 800 × *g* at 4 °C for 8 min[11] (in an Eppendorf centrifuge). The supernatants were subjected to 12% SDS-PAGE and analyzed by densitometry using the TINA 2.07d software. The presented data are the mean and standard deviation of three independent experiments.

2.7. Actin polymerization

The polymerization of actin was followed as an increase in fluorescence of the pyrene-labeled G-actin,[9] which was added to unlabeled G-actin in 10–15%. The time course of pyrene-labeled actin polymerization was monitored by measuring the fluorescence increase (at 365 nm excitation and 386 nm emission wavelengths) in a PTI spectrofluorometer (Photon Technology Industries, South Brunswick, NJ).

2.8. Transglutaminase (TGase) crosslinking

Unless stated otherwise, actin, LL-37, f-LL-37, FK-13, KR-12, KR-10 and 0.2 mg ml^{-1} TGase were mixed together simultaneously and incubated at room temperature for 90 min. The reaction was stopped by the addition of protein sample buffer (PSB) and incubation in boiling water for 5 min. The samples were subjected to 12% SDS-PAGE, visualized by Coomassie blue, or rhodamine fluorescence and evaluated by densitometry.

2.9. Statistics

Unless specified, all presented data are the mean and standard deviation of three independent experiments performed in triplicate. All presented SDS-gels are representative of three independent experiments. The Student's *t*-test was used for the calculation of the *P*-value.

3. Results

3.1. Actin protects the LL-37 core peptide FK-13 from bacterial proteolysis

A previous report stated that the antimicrobial activity of the FK-13 and the KR-12 core peptides was practically the same as their LL-37 parent molecule, when tested against the *Escherichia coli* strain K12. The activity of the C-terminal truncated peptide KR10 however, was much weaker with an MIC of 160 μM.[21] Here, we measured the antimicrobial activity of the core peptides against *Bacillus subtilis* PY79 (Table 1), which is much more sensitive to LL-37 (MIC of 1.1 μM) than the previously tested *E. coli*. Under our tested conditions, the core peptides had considerable antimicrobial activity, but it was lower than that of LL-37. Of the core peptides, FK-13 was the most active against *B. subtilis* (MIC of 3.3 μM) and the activity of the peptides decreased in the order FK-13 > KR-12 > KR-10 (Table 1).

To further understand the antimicrobial activity of these core peptides, we evaluated the effect of presence or absence of 100 mM NaCl on their antimicrobial activity (Table 2). In the absence of salts, the core peptides, KR-12 and FK-13, are nearly as active as LL-37 against *E. coli* K12 with MIC in the range of 10–20 μM. In the presence of 100 mM NaCl, however, the antimicrobial activity of KR-12 dropped by eight fold, whereas the activities of FK-13 and LL-37 were essentially unchanged. Because previous studies suggest membrane targeting of these peptides,[10,21] electrostatic interactions occupy a large portion of such interactions in the case of KR-12.

The effect of bacterial proteolysis on the antimicrobial activity of FK-13 was also tested (Fig. 1). The peptide was found to be susceptible to the protease of the oral anaerobic periodontal pathogen *P. gingivalis* because it completely lost its ability to inhibit the growth of *B. subtilis* after treatment with the growth medium supernatant prepared from *P. gingivalis*. However, F-actin protected the antimicrobial activity of FK-13 against inactivation by the *P. gingivalis* supernatant (Fig. 1) in a similar manner that the antimicrobial activity of LL-37 was protected.[18]

Table 2 The MIC (μM) of LL-37 and its core peptides against *E. coli* K12, with and without salt[a]

Peptide	No salt	100 mM NaCl
LL-37	10	10
FK-13	10–20	20
KR-12	10–20	80–160
KR-10	>160	>160

[a] Antibacterial assays in tryptic soy broth (TSB).

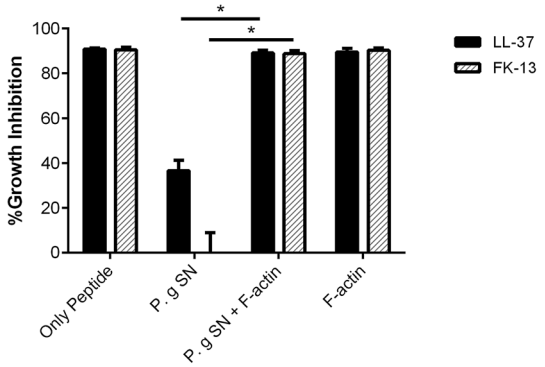

Fig. 1 F-actin protects and enables the antibacterial activity of FK-13 and LL-37 in the presence of *P. gingivalis* proteases. Growth inhibition of *B. subtilis* by 3.3 μM FK-13 or 1.1 μM LL-37 treated or not with *P. gingivalis* supernatant (*P. g.* SN), in the presence or absence of 1 : 3 molar ratio of F-actin. *Represents $P < 0.05$ in Student's *t* test.

3.2. FK-13, KR-12 and KR-10 polymerize G-actin to filaments

Previously, low micromolar concentrations of LL-37 were found to polymerize G-actin to F-actin rapidly and efficiently.[17] In this work, we studied the ability of the core peptides to polymerize actin in comparison to that of LL-37 (Fig. 2). The polymerization of 4 μM G-actin to F-actin by 8 μM LL-37 was fast and extensive; the polymerization by 50 μM FK-13 was much slower and less complete, while very weak and slow polymerization was obtained by 50 μM KR-12 and 100 μM KR-10. Actin is polymerized by mono, di, and polyvalent cations and the efficiency of polymerization is dependent on the concentration and number of positive charges of the cation. This explains the difference in the ability of LL-37 to polymerize actin compared to its core peptides. This is because LL-37 has six net positive charges, FK-13 and KR-12 have five and KR-10 has only four net positive charges (Table 1). However, we found it surprising that there is a big difference in the ability of FK-13 and KR-12 to polymerize actin, because both have the same number of net positive charges.

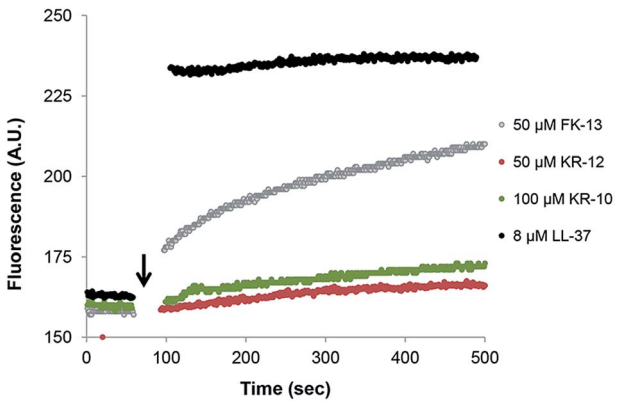

Fig. 2 Polymerization of 4 μM CaATP-G-actin by LL-37, FK-13, KR-12 and KR-10. Pyrene-labeled G-actin was polymerized as described in Materials and methods. Arrow in the graph indicates the time of peptides addition.

3.3. FK-13 and KR-12 but not KR-10 bundle actin filaments

Polycations bundle F-actin.[11] The extent of bundling increases with the number of net positive charges and concentration of the polycations. Bundling is mostly based on electrostatic interactions between the negatively charged actin and the polycation, however, specific hydrophobic interactions can significantly contribute to the bundling ability of the polycation.[18] This is the case with LL-37 that has a high affinity to actin as shown by surface plasmon resonance measurements (ref. 17 and Table 1) and a low sensitivity of its actin bundling activity to an increase in ionic strength (Fig. 3 and ref. 17). Using surface plasmon resonance, we compared the affinity of actin binding to the FK-13, KR-12 and KR-10 fragments with the affinity of its binding to LL-37. The affinity of the LL-37 core peptides to G-actin is about two magnitudes lower than that of the parent peptide (Table 1). The G-actin binding affinity of the LL-37 fragments decreases in the order KR-12 > FK-13 > KR-10 (Table 1). Thus, the number of hydrophobic residues on the hydrophobic surfaces of these helical peptides[21] could also play a role (Table 1) in actin binding.

The F-actin bundling activity of LL-37 and that of its core peptides was measured both at low ionic strength and in the presence of 100 mM NaCl (Fig. 3). At low ionic strength, about 80 percent of 4 μM F-actin was bundled upon addition of 6 μM LL-37 while the addition of 25 μM FK-13 or KR-12 was needed to achieve the same degree of bundling and no bundling was obtained even by 130 μM of KR-10 (Fig. 3A, panel b). The presence of 100 mM NaCl did not significantly affect the degree of actin bundling by LL-37 but essentially abolished actin bundling by FK-13 and KR-12 (Fig. 3B). These results clearly indicate that there is no significant hydrophobic interaction between the LL-37 core peptides and actin and that their reaction with actin is mediated mostly by electrostatic interactions. However, the electrostatic interaction of the core peptides with actin is also weaker than that of LL-37, due to their reduced net positive charge (+5), compared to that of the parent peptide (+6). The net positive charge of KR-10 that is even lower (+4), explaining the inability of KR-10 to bundle F-actin readily at low ionic strength.

3.4. FK-13, KR-12 and KR-10 can be cross linked with actin

Transglutaminase (TGase) catalyzes the formation of a cross-linking bond between a glutamine and a lysine residue. Treatment of LL-37 and G-actin with transglutaminase leads to the formation of LL-37–actin, LL-37–actin dimer, and LL-37 dimer–actin cross-linked products (ref. 18 and Fig. 4). Our previous mass spectrometry and limited proteolysis results revealed that LL-37 is cross-linked by TGase to the His40–Lys50 segment of the DNase-I binding loop (D-loop) of actin.[18] Here, we studied cross-linking of FK-13, KR-12 and KR-10 to G-actin and compared it to that of LL-37 (Fig. 4). Because of the low molecular weight of the core peptides, we could not observe separate actin–peptide cross-linked bands with the three core peptides, as observed with the larger LL-37, (Fig. 4). However, the thickening of the actin band upon transglutaminase treatment clearly indicates the formation of actin–peptide cross-

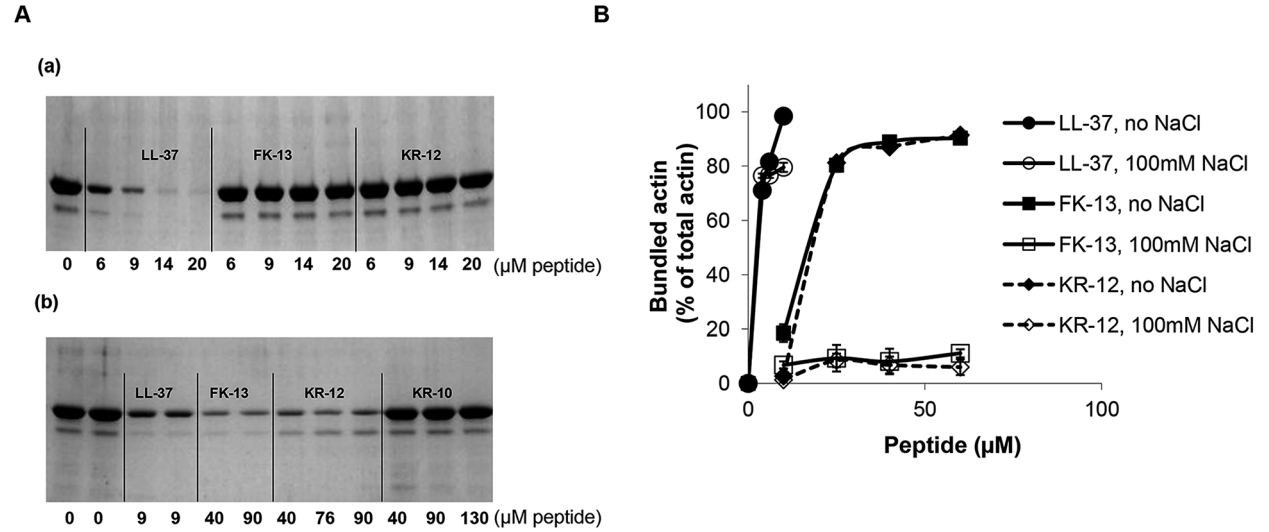

Fig. 3 Bundling of MgF-actin by LL-37, FK-13, KR-12 and KR-10 at high and low ionic strength. MgF-actin (4 μM) was sedimented at 20 800 × *g* for 8 min after incubation with LL-37, FK-13, KR-12 and KR-10. (A) Representative SDS-PAGE of the supernatant of actin incubated with low (a) and high (b) concentrations of LL-37, FK-13, KR-12 and KR-10. (B) Quantitative evaluation of the densitometry results of SDS-gels.

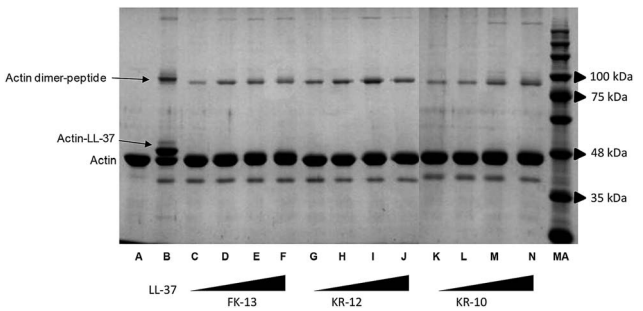

Fig. 4 Cross-linking LL-37, FK-13, FK-12 and KR-10 to 6 μM CaATP-G-actin by transglutaminase. Cross linking was carried out as described in the Experimental procedures. Representative SDS-gel. Lanes: (A), actin only; (B), actin and 9 μM LL-37; (C), actin and 9 μM FK-13; (D), actin and 15 μM FK-13; (E) actin and 22 μM FK-13; (F), actin and 30 μM FK-13; (G) actin and 9 μM KR-12; (H), actin and 15 μM KR-12; (I), actin and 22 μM KR-12; (J) actin and 30 μM KR-12; (K), actin and 9 μM KR-10 (L) actin and 15 μM KR-10; (M) actin and 22 μM KR-10; (N) actin and 30 μM KR-10; (MA), marker.

links (Fig. 4). Moreover, the formation of peptide–actin dimer cross-links was observed with all of the peptides studied even at the lowest concentration used, which clearly indicates the formation of actin–peptides cross-links. In order to quantitatively evaluate the actin–peptides cross-linking, we studied the competition between the three peptides and rhodamine-labeled fluorescent LL-37 (f-LL-37) for cross-linking to G- (Fig. 5A) and F-actin (Fig. 5B). All of the three peptides successfully competed with 6 μM f-LL-37 for G-actin cross-linking in the order of KR-12 > KR-10 > FK-13 (Fig. 5A) and for cross linking to F-actin in the order KR-12 > FK-13 > KR-10 (Fig. 5B). Interestingly, KR-10, which essentially does not polymerize or bundle actin, is a more efficient competitor of f-LL-37 for cross-linking to G-actin than FK-13. It is possible that the N-terminal phenylalanine residue of FK-13 somehow inhibits the proper peptide–G-actin alignment needed for the cross-linking. The ability of these core peptides to compete with f-LL-37 for actin-binding is proportional to their protein binding potential (or Boman index in Table 1).[2] With F-actin, less cross-links were obtained than with G-actin, which may be due to the rigid, highly ordered structure of the D-loop in F-actin that renders the cross-link formation more difficult. In both G- and F-actin about 10 fold excess concentrations of core peptides over f-LL-37 were needed to extensively compete out LL-37 from the cross-link, which indicates that the affinity of the core peptides to actin is much less than that of the parent LL-37.

4. Discussion

Host defense peptides in general and LL-37 in particular, have a key role in the maintenance of systemic and oral health.[25] Bacterial pathogens including the periodontal pathogen *P. gingivalis* evade host defense peptides by proteolytic inactivation. It was found earlier that the host defense peptide LL-37 interacts with the structural protein actin.[17,24] We showed that this interaction protects LL-37 from proteolysis and enables the antimicrobial activity of the peptide[18] in the presence of proteases. Structural–functional studies revealed that fragments of LL-37, the FK-13 and KR-12 so called core peptides have the same or almost the same antimicrobial activity as the parent molecule against *E. coli*,[21] suggesting a therapeutic potential. Here unsurprisingly, we show that the tested, most potent core peptide, FK-13 is susceptible to bacterial proteolysis (by *P. gingivalis*). However, similar to LL-37 the antimicrobial action of FK-13 is protected by actin from microbial proteolytic inactivation. Next, we studied how these core peptides and the shorter, less active KR-10 peptide interact with actin in comparison to LL-37. We found that the core peptides, which bind actin, can be cross-linked to actin, and that they can polymerize and bundle actin.

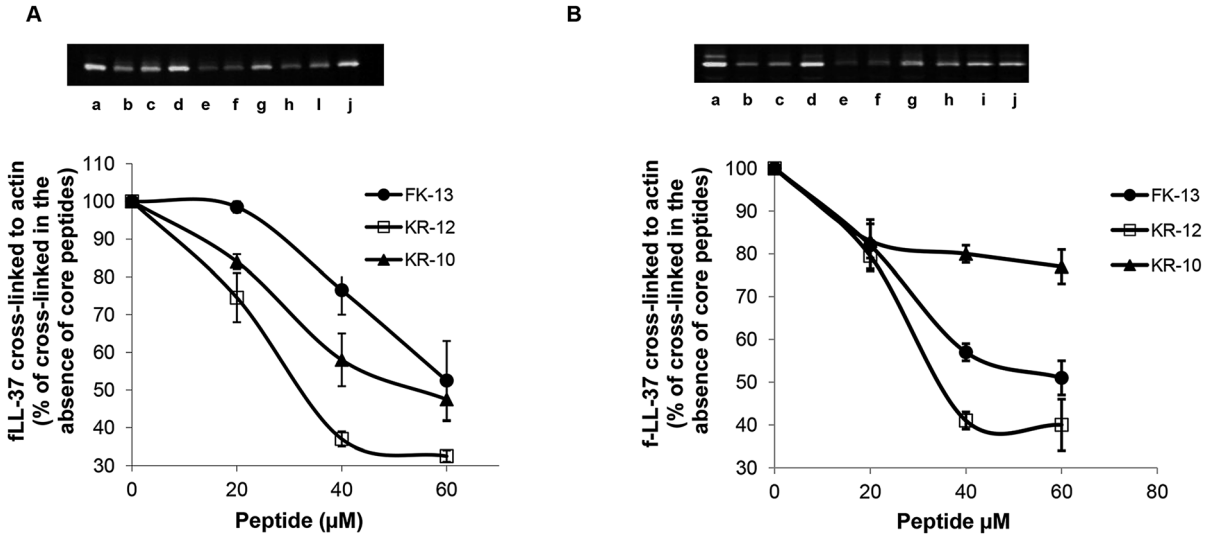

Fig. 5 Competition between 6 μM f-LL-37 and 0–60 μM FK-13, KR-12 and KR-10 for cross-linking to CaATP-G-actin (A) or MgF-actin (B) by transglutaminase. Cross linking was carried out as described in the Experimental procedures. Upper panels display representative fluorescent SDS-gels. Lanes: (a) actin, f-LL-37; (b) actin, f-LL-37, 60 μM FK-13; (c) actin, f-LL-37, 40 μM FK-13; (d) actin, f-LL-37, 20 μM FK-13; actin, f-LL-37, 60 μM KR-12; (e) actin, f-LL-37, 40 μM KR-12; (f) actin, f-LL-37, 20 μM KR-12; (g) actin, f-LL-37, 60 μM KR-10; (h) actin, f-LL-37, 40 μM KR-10; (i) actin, f-LL-37, 20 μM KR-10; (j) lower panel quantitative assessment of SDS-gels.

It is of interest to compare the antimicrobial activity and the interaction with actin of the two core peptides FK-13 and KR-12. The antimicrobial activity against *B. subtilis* and the actin polymerization capability of FK-13 is greater than that of KR-12. The F-actin bundling activity of the two core peptides are equal, but the actin binding affinity of KR-12 (according to plasmon resonance measurements), and the ability of KR-12 to cross-link with actin is higher than that of FK-13. The better antimicrobial activity (lower MIC) of FK-13 compared to KR-12 might result from a better interaction with the membrane of *B. subtilis*. In the case of the Gram-negative *E. coli*, FK-13 and KR-12, both with a net charge of +5, showed similar activity. However, the activity of KR-12 is much more sensitive to 100 mM NaCl than FK-13, suggesting the importance of electrostatic interactions (Table 2).

It is not easy to explain the different behavior of the two peptides in the various interactions with actin. The only structural difference between them is that FK-13 contains an additional phenylalanine (Phe) residue that must cause the differences in the behavior of the two peptides with actin. It is possible that this phenylalanine promotes actin polymerization while it inhibits the proper alignment (binding) of FK-13 to actin in the plasmon resonance and in the cross-linking experiments. On the other hand, for actin bundling, only the number of the net positive charges is important, which is equal (+5) in the two core peptides and therefore, there is no difference in their ability to bundle actin. KR-10 has much weaker antimicrobial, actin-polymerization and actin-bundling activities compared to the core peptides because it has less (+4) net positive charges than FK-13 and KR-12. However, it has a greater ability to cross-link with G-actin compared to FK-13 probably because its alignment to actin is not inhibited by the bulky Phe residue.

5. Conclusions

Using four different experimental setups, we demonstrated that the LL-37 core peptides associate with actin, however, their affinity to actin is considerably less than that of LL-37. Their association with actin, which is very ionic strength sensitive, is mainly based on electrostatic interactions (unlike the interactions of actin with LL-37, where the hydrophobic interactions have a significant role). Interestingly, FK-13 and LL-37 have antimicrobial activity at a physiological salt concentration, while KR-12 does not. Most importantly, the antimicrobial activity of the FK-13 core peptide is protected by actin against *P. gingivalis* protease in a similar manner to that of LL-37, supporting the earlier suggestions of the effective, economic, therapeutic use of FK-13.

Acknowledgements

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