Conformation-dependent Antibacterial Activity of the Naturally Occurring Human Peptide LL-37*

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Jan Johansson‡, Gudmundur H. Gudmundsson§, Martín E. Rottenberg§, Kurt D. Berndt‡, and Birgitta Agerberth‡¶
From the ‡Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-17177 Stockholm, Sweden and §Microbiology and Tumorbiology Center, Karolinska Institutet, S-17177 Stockholm, Sweden

The influence of ion composition, pH, and peptide concentration on the conformation and activity of the 37-residue human antibacterial peptide LL-37 has been studied. At micromolar concentration in water, LL-37 exhibits a circular dichroism spectrum consistent with a disordered structure. The addition of 15 mM HCO$_3^-$, SO$_4^{2-}$, or CF$_3$CO$_2^-$ causes the peptide to adopt a helical structure, with approximately equal efficiency, while 160 mM Cl$^-$ is less efficient. A cooperative transition from disordered to helical structure is observed as the peptide concentration is increased, consistent with formation of an oligomer. The extent of $\alpha$-helicity correlates with the antibacterial activity of LL-37 against both Gram-positive and Gram-negative bacteria. Two homologous peptides, FF-33 and SK-29, containing 4 and 8 residue deletions at the N terminus, respectively, require higher concentrations of anions for helix formation and are less active than LL-37 against *Escherichia coli* D21. Below pH 5, the helical content of LL-37 gradually decreases, and at pH 2 it is entirely disordered. In contrast, the helical structure is retained at pH over 13. The minimal inhibitory concentration of LL-37 against *E. coli* is 5 $\mu$M, and at 13–25 $\mu$M the peptide is cytotoxic against several eukaryotic cells. In solutions containing the ion compositions of plasma, intracellular fluid, or interstitial fluid, LL-37 is helical, and hence it could pose a danger to human cells upon release. However, in the presence of human serum, the antibacterial and the cytotoxic activities of LL-37 are inhibited.

During the past decade, the widespread appearance of naturally occurring antibacterial peptides has been firmly established. Their abundance, tissue distribution, and in vitro activity suggest an essential role in biological defense systems (1). In mammals, antibacterial peptides such as defensins have been located in circulating leukocytes, where they are a part of the intracellular bactericidal machinery (2). Other peptides, such as those belonging to the cathelicidin family, are released upon stimulation and exert their activity extracellularly (3). Recently, several broad spectrum bactericidal peptides have been found to be expressed or induced at surface epithelia, probably providing an effective barrier for invading bacteria (4–7).

Sequence comparison of gene-encoded antibacterial peptides from vertebrate reveals a pronounced heterogeneity. In general, they can be divided into four major groups according to composition and secondary structure. One group, which includes the defensins, is folded into an antiparallel $\beta$-sheet structure, containing three disulfide bridges (2). A second group, which includes cecropins and magainins, exhibits an $\alpha$-helical structure (8, 9). A third group comprises peptides that form loop structures with one or more disulfide bridges, such as batocin (10). The fourth group comprises peptides with a high content of specific amino acid, such as the proline-arginine-rich peptide PR-39 (11) and the tryptophan-rich peptide indolicidin (12). Despite these very diverse structural motifs, many of these peptides are membrane-active, containing an amphipathic secondary structure (13, 14). They kill bacteria mainly by lysis, and some are also cytotoxic to eukaryotic cells. However, the nonlytic PR-39 adopts a nonamphiphilic polyproline-helix at low temperature (15) and kills bacteria by interrupting both DNA and protein synthesis (16).

The human cathelicidin peptide LL-37 was originally predicted from a cDNA clone, and the putative active peptide was synthesized as FA-LL-37 (14). Later, the mature active peptide LL-37 (two residues shorter at the N-terminal end than the predicted peptide) was isolated from degranulated granulocytes (17). LL-37 exhibits moderate antibacterial activity in *Luria-Berti*=n LB) medium, but upon the addition of medium E (a salt medium used for culturing *Escherichia coli*) a pronounced increase of the antibacterial activity was noticed. This enhancement of the activity was shown to correlate with induction of an $\alpha$-helical structure (14).

We have found the $\alpha$-helical conformation of LL-37 to be anion-, pH-, and concentration-dependent. The extent of $\alpha$-helical content correlates well with the observed antibacterial activity. The minimal inhibitory concentration (MIC) of LL-37 against *E. coli* D21 was determined to 5 $\mu$M, and at 3–5 times this concentration, the peptide also exhibits cytotoxic activity toward eukaryotic cells. This means that LL-37, when released, could cause host cell damage. A mechanism for protection from such potentially harmful effects appears to be in place in the circulation, since we find that the cytotoxic activity of LL-37 is inhibited by human serum.

EXPERIMENTAL PROCEDURES

Materials—Porcine and human sera were purchased from Sigma and from the Department of Transfusion Medicine and Clinical Immunology, Karolinska Hospital (Stockholm, Sweden), respectively. The com-

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‡ To whom correspondence should be addressed: Birgitta Agerberth, Dept. of Medical Biochemistry and Biophysics, Chemistry I, Doktorsringen 9A3, Karolinska Institutet, S-17177 Stockholm, Sweden. Tel.: 46-8-7287699; Fax: 46-8-337462; E-mail: Birgitta.Agerberth@mbb.ki.se.

¶ The abbreviations used are: LB, Luria-Bertani; FDA, fluorescein diacetate; MIC, minimal inhibitory concentration; PBL, peripheral blood leukocytes; HPLC, high pressure liquid chromatography.
position of medium E (18) is 0.8 mM MgSO4, 9.6 mM citric acid, 57.4 mM K2HPO4, and 16.7 mM NaHCO3, 0.6 mM MgCl2, 1.3 mM CaCl2, 3.9 mM KCl; for interstitial fluid, 117.0 mM NaCl, 27.0 mM NaHCO3, 0.6 mM MgCl2, 1.1 mM CaCl2, 4.0 mM KCl; for intracellular fluid, 70.0 mM K2HPO4, 12.0 mM NaHCO3, 17.0 mM MgCl2, 2.0 mM CaCl2 (19). All physiological salt solutions were adjusted to pH 7.3 with 2 M HCl.

Peptide Synthesis—The amino acid sequences of LL-37 and the N-terminally truncated forms FF-33 and SK-29 are shown in Fig. 1. Peptide synthesis was performed with an Applied Biosystems model 430A peptide synthesizer using standard solid-phase procedures (for review, see Ref. 20). Starting from t-butyloxycarbonyl-Ser(benzyl)-OCH2CONH2, the peptides were cleaved from the resin with liquid hydrogen fluoride/anisole/methylsilicic acid (10:1:1, v/v) for 60 min at 0 °C. The cleavage products were washed with diethyl ether to remove scavengers and protecting groups, extracted in 30% acetic acid, and lyophilized. The peptides were further purified by HPLC (Waters) on a reversed phase Vydac C18 column (The Separation Group) using a linear gradient of acetonitrile (15–60% in 40 min) in 0.1% trifluoroacetic acid. The molecular masses were determined with a matrix-assisted laser desorption/ionization instrument (Lasermat 2000, Finnigan MAT) and were in all cases in agreement with the calculated masses.

CD Spectroscopy—CD spectra were recorded on either a Jasco J-720 (Jasco Inc.) or an Aviv 62DS (Aviv Associates Inc.) spectropolarimeter. All spectra were recorded at 0.5 nm point resolution, and data were reported as differences in molar absorptivities (Δε = εl – ε0) of the backbone amide bond (ν = 1 cm−1). Peptide concentration was 40 μM unless otherwise noted. Helical content was estimated by obtaining the average of the following values: percentage of helix = Δ(208) – 1212/Δ(222) – 11.22 (21) and percentage of helix = Δ(208) – 0.91/Δ(222) – 11.82 (22). For determination of the concentration dependence of the peptide CD, concentrations between 10−3 and 10−5 M were measured in cuvettes with path lengths between 0.1 and 50 mm.

Antibacterial Activity—An inhibition zone assay in thin (1-mm) agarose plates seeded with E. coli D21 or Bacillus megaterium Bm11 was used. 1% (w/v) agarose in LB broth was supplemented with different single salt solutions or medium E. Antibacterial activity in “salt-free” medium was determined by omitting NaCl from the LB medium. Bacteria were grown overnight on agar plates containing streptomycin (100 μg/ml) and were then inoculated in LB medium. Bacteria were added to the agarose mixture just before plating to a concentration of 6 × 10^6 cells/ml. Small wells (3-mm diameter, 3-μl volume) were punched out of the plates, and a dilution series of a given peptide (starting with 5 μg/μl in water) was applied. For serum inhibition studies, LL-37 was dissolved at a concentration of 1 μg/μl in water, human serum, or porcine serum, with 3 μl applied of each sample. After overnight incubation at 30 °C, the diameters of bacteria-free zones were measured.

For the MIC value, E. coli D21 was grown to late log phase. Approximately 2000 bacteria were incubated in LB supplemented with medium E and different peptide concentrations (ranging from 20 to 0.31 μM in 1:1 dilution steps) for 3 h. The lowest concentration inhibiting bacterial growth was taken as the MIC value.

Analysis of Cellular Viability by Flow Cytometric Analysis—The cytotoxic effect of LL-37 was determined by the ability of intracellular eukaryotic esterases to hydrolyze fluorescein diacetate (FDA) to free fluorescein, followed by flow cytometric analysis (23). LL-37 was incubated with either trypomastigote forms of Trypanosoma cruzi (Tulahuen strain (24)), obtained from the culture supernatant of L-929 infected cells, human peripheral blood leukocytes (PBL), or the T-cell line MOLT. Cells were resuspended in RPMI 1640 medium containing 5% fetal calf serum, penicillin, and streptomycin, in the absence or presence of indicated dilutions of LL-37, at 37 °C and 5% CO2. Serum inhibition of cytotoxicity was analyzed by using serial dilutions of human serum (40–0%), 50 μM LL-37, and PBL. After 12–16 h, cells were centrifuged, and the pellet was washed once in phosphate-buffered saline. Cells were resuspended to 2 × 10^6/ml and incubated with 1 mg/ml FDA in phosphate-buffered saline, (a 100-fold dilution from a stock FDA in acetone). After incubating 15 min at 37 °C, cells were washed once in phosphate-buffered saline and fixed in 2% paraformaldehyde for 10 min at room temperature, and 10^5 events were then analyzed in a FACScan flow-cytometer (Beckton & Dickinson). The percentage of nonviable cells was determined as the number of events showing fluorescein staining after LL-37 incubation as compared with control cells incubated with RPMI 1640 medium alone.

RESULTS
Concentration-, Anion-, and pH-dependent Transition from Disordered to Helical Structure of LL-37—In 20 mM SO4− 2 at a concentration of 10−3 M, the CD spectrum of LL-37 is dominated by double minima (222 and 208 nm) and a single maximum (195 nm) characteristic of an α-helical secondary structure (25) (Fig. 2A). At this concentration, the α-helical content is estimated to be 50%. This helical conformation is lost in a cooperative fashion upon dilution of the peptide in 20 mM SO4− 2 buffer. The existence of an isodichroic point (204 nm) is consistent with a two-state helix-coil equilibrium (26). At the lowest concentration measured (10−7 M), the CD spectrum of LL-37 has lost essentially all α-helical character and now contains a single pronounced minimum at about 200 nm. The position of
the minimum undergoes a blue shift with decreased peptide concentration (Fig. 2A). This is expected as the small amount of remaining helical structure is lost and further supports the notion of a largely disordered peptide at concentrations below \(10^{-6}\) M. Taken together, this highly cooperative concentration-dependent helix-coil equilibrium is highly reminiscent of the monomer-oligomer transitions common to peptide sequences capable of forming amphipathic \(\alpha\)-helices (e.g. melittin; Ref. 27). Preliminary analysis of the concentration-dependent CD and sedimentation equilibrium experiments indicates that a significant portion of the helical form of LL-37 is a tetramer (data not shown). We cannot at present rule out the presence of higher molecular weight oligomers.

At a concentration of 40 \(\mu\)M, the CD spectrum of LL-37 in water exhibits a minimum around 200 nm, which is indicative of a highly disordered structure (see Fig. 5A). Upon the addition of 15 mM \(\text{Na}_2\text{SO}_4\), \(\text{NaHCO}_3\), or \(\text{NaCF}_3\text{CO}_2\), a conformational change occurs, as evidenced by spectra containing minima at 208 and 222 nm and a maximum around 195 nm (Fig. 2B). Based on these spectra, the helicities in the presence of these salts are similar (Fig. 2B) and are estimated to be about 40%. The addition of up to 40\% (v/v) trifluoroethanol to LL-37 in water results in approximately 30\% helical content (data not shown). 15 mM NaCl affects the structure of LL-37 to a very limited extent (Fig. 2B), and replacement of \(\text{Na}^+\) with \(\text{Mg}^{2+}\) does not have any effect on the structural transitions observed, which shows that the structural changes are predominantly caused by the \(\text{SO}_4^{2-}\), \(\text{HCO}_3^-\), and \(\text{CF}_3\text{CO}_2^-\) anions.

Given the clear cut effects of the anion composition and concentration on the secondary structure of LL-37, we next examined the peptide conformation in solutions with ion compositions similar to those of various physiological environments. Fig. 3 shows that LL-37 adopts an \(\alpha\)-helical conformation virtually identical to that in medium E in solutions containing ion compositions that mimic those of human plasma, interstitial fluid, and intracellular fluid.

The helicity of LL-37 in salt solutions as inferred from CD measurements is reduced at low pH. Below pH 5, a transition to a disordered conformation is evident, and at pH 2–3, the CD spectrum is practically identical to that in water (Fig. 4). The original \(\alpha\)-helical structure is regained upon raising the pH (not shown). In contrast, the helical content is retained at pH values over 13 (Fig. 4), and the addition of 50 mM NaOH to LL-37 dissolved in water causes a transition to a helical conformation (not shown).

**The Helical Content Correlates with Antibacterial Activity**—Fig. 5A shows the CD spectra of LL-37 (40 \(\mu\)M) in water and after the addition of various salts. The addition of medium E or 84 mM \(\text{SO}_4^{2-}\) results in the most pronounced helix formation. 160 mM Cl\(^-\) induces formation of helical structure, but the helical content is only about half of that observed with medium E or 84 mM \(\text{SO}_4^{2-}\) (Fig. 5A). The antibacterial activity of LL-37 in the presence of these ions was determined using an inhibition zone assay. The activity toward the Gram-positive bacterium *B. megaterium* Bm11 closely correlates with the helical content (Fig. 5B). The activities in medium E, 5 and 84 mM \(\text{Na}_2\text{SO}_4\), and 12 mM \(\text{NaHCO}_3\) are similar and significantly higher than that in 160 mM NaCl, which in turn is higher than the activity observed in “salt-free” medium (Fig. 5B). The same trend is observed for the activity toward the Gram-negative bacterium *E. coli* D21 (Fig. 5C), except that the activity in 12 mM \(\text{NaHCO}_3\) is reduced, probably due to the different compositions of the membranes for Gram-positive and Gram-negative bacteria. Finally, the MIC value for LL-37 against *E. coli* D21 in LB supplemented with medium E was found to be approximately 5 \(\mu\)M.

Further support for a direct relation between helical content and antibacterial activity comes from analyses of N-terminally truncated versions of LL-37. The helical content of LL-37, FF-33, or SK-29 each increases with increasing \(\text{SO}_4^{2-}\) concentration (Fig. 6A). All peptides show an isodichroic point at 203–204
nm upon salt titration (not shown), consistent with a two-state helix-coil transition. The shorter peptides require significantly higher concentrations of SO$_4^{2-}$ for induction of maximal helical content, approximately 300 mM for FF-33 and SK-29 compared with about 30 mM for LL-37. This is reflected in their respective activities against *E. coli* D21 in 5 mM Na$_2$SO$_4$, where LL-37 is more active than FF-33 or SK-29 (Fig. 6B). In medium E, LL-37 and FF-33 are equally active and significantly more active than the shorter SK-29 peptide (Fig. 6C).

Correlation of pH with the antibacterial activity could not be determined, since the bacterial test strains do not grow well at extremes of pH. However, values were obtained for *E. coli* D21 at pH 4.3, where the inhibition zones were, depending on the peptide concentration, 40–85% of the zones obtained at pH 7.

**Cytotoxic Effects of LL-37**—The cytotoxic effect of LL-37 was studied by FDA incorporation using three different types of eukaryotic cells: trypomastigotes of the protozoan parasite *T. cruzi*, a T lymphocyte cell line (MOLT), and PBL. For these cell types, toxicity is clearly observed at 13–25 μM of LL-37, and gradually increases at higher concentrations (Fig. 7).

**The Antibacterial and Cytotoxic Activities of LL-37 Are Inhibited in Serum**—Our data show that the active helical conformation of LL-37 is cytotoxic to eukaryotic cells in the ion compositions and at pH values that are encountered under physiological conditions. Thus, LL-37, if released extracellularly, could be harmful to human cells *in vivo*. However, such potential effects are apparently attenuated by one or several factors present in human serum, since the cytotoxic effect is reduced when human serum is included in the assay. In the absence of serum, 50 μM LL-37 results in 81% nonviable cells, while in the presence of 40% serum, 38% nonviable cells are found. In addition, the antibacterial activity of LL-37 is lost completely when the peptide is first dissolved in human serum and significantly reduced in porcine serum (Fig. 8). We interpret the bactericidal activity of the peptide dissolved in water (Fig. 8) as being the result of the conformational inducing effects of medium E in the agarose plate. Control experiments indicate that the observed inhibition is not likely to be caused by proteolytic degradation of LL-37. After incubation in human plasma for 20 h at 25 °C, the peptide elutes at the identical position as untreated LL-37 upon reversed phase HPLC, and the mass value determined by matrix-assisted laser desorption/ionization time of flight mass spectrometry is identical to that of intact LL-37.
DISCUSSION

Salt-, pH-, and Concentration-dependent Folding of LL-37—
The cooperative concentration-dependent α-helix formation of LL-37 is consistent with a system in which largely unfolded monomeric peptide (the dominant form at \( \sim 10^{-5} \) M) is in equilibrium with an α-helical oligomer form. This is indeed the expected behavior of an amphiphilic α-helix such as LL-37 (Figs. 1 and 2). The driving force of oligomerization and concomitant α-helix formation is then expected to be due largely to the hydrophobic effect, i.e., efficient removal of the apolar face from contact with the solution to form the interior of the oligomer, leaving the polar face exposed. In this context, the effect of some ions (SO\(_4^{2-}\), HCO\(_3^-\), CF\(_3\)CO\(_2^-\) and to a significantly lesser extent Cl\(^-\)) in promoting helix formation in LL-37 can be rationalized from their ability to “salt out” nonpolar groups, commonly referred to as the Hofmeister effect (see Refs. 28 and 29). The salting out effect is often a manifestation of reduced solubility at high salt concentrations (\( > 1 \) M), hence the name. However, the salting out of apolar groups is thought to result from the effect of ions on the surface tension of water. By increasing the surface tension of water, the exposure of hydrophobic surface becomes more unfavorable.

Ionic interactions along the polar face of the amphiphilic helix also appear to play a role in the conformation of LL-37. Salt bridges in helices, formed by location of residues with acidic and basic side chains, respectively, in positions \( i \) and \( i + 3 \) or \( i + 4 \), which are close in a helical conformation, specifically stabilize the helical conformation (30). LL-37 contains

![Figure 6. Folding and activity of N-terminally truncated forms of LL-37.](image)

**A**. CD at 222 nm as a function of SO\(_4^{2-}\) concentration for LL-37, FF-33, and SK-29 at a peptide concentration of 40 μM, 20–25 °C, pH 6.  
**B**, the antibacterial activity of the peptides LL-37, FF-33, and SK-29 in LB medium supplemented with 5 mM Na\(_2\)SO\(_4\) against *E. coli* D21.  
**C**, same as B except the LB was supplemented with medium E.

![Figure 7. Cytotoxic effect of LL-37 against eukaryotic cells.](image)

The toxic effect for different peptide concentrations was measured by FDA staining. The cell types are *T. cruzi*, the T lymphocyte cell line MOLT, and PBL.

![Figure 8. Inhibition of antibacterial activity of LL-37 in serum.](image)

An inhibition zone assay on *E. coli* D21 in the presence of medium E with the human peptide LL-37 and the porcine nonlytic peptide PR-39. The peptides were dissolved at a concentration of 1 μg/μl in water, human serum, or porcine serum as indicated. 5 μl were loaded in each well. The diameter of the inhibition zone for LL-37 in water is 10.5 mm.
nine such potential ion pairs (Fig. 1). In addition, LL-37 contains seven locations where residues with basic side chains are located both in positions i and i + 3 or i + 4, but no instances where two negative charges are in such positions (Fig. 1). It is likely that the anion-induced folding of LL-37 into a helical structure at near neutral pH (Figs. 2B and 5A) is at least in part caused by reducing repulsive forces between positively charged residues located in the seven (i, i + 3/i + 4) positions. The unfolding of α-helical LL-37 at low pH (Fig. 4) could be brought about by protonation of acidic side chains with concomitant losses of stabilizing complimentary (i, i + 3/i + 4) side chain ion pairs, since destabilizing interactions between positive residues are expected to be largely unaffected at low pH. This in turn suggests that, in the present case, i, i + 3/i + 4 ion pairs are more helix-stabilizing than the corresponding hydrogen bonds between protonated Asp or Glu side chains and positively charged side chains. In contrast, Marques and Baldwin (31) found that, in 16–17-residue model peptides, Glu–Lys hydrogen bonds are roughly as effective as the Glu–Lys salt bridge in stabilizing the helix. However, an important caveat is that in their model system all peptides were shown to be monomeric, whereas LL-37 is clearly an oligomer when helical. Finally, at pH > 13, where the side chains of Lys and Arg are uncharged, complimentary side chain ion pairs are again lost, but so are repulsive forces between basic side chains. This does not cause a net destabilization of the LL-37 helical fold (Fig. 4).

Conformational Requirement for Antibacterial Activity—The helical, oligomeric conformation of LL-37 is apparently a requirement for activity, since the highest antibacterial activity correlates to maximal helical content, while intermediate and low activities correspond to less helical content and disordered secondary structure (Fig. 5). This indicates that optimal antibacterial activity of LL-37 requires an oligomeric α-helical structure prior to interacting with the bacterial membrane. Hence, for LL-37 the bacterial membrane alone cannot be a major determinant for folding into an active conformation, as has been claimed for the cecropins (32).

In cystic fibrosis, the innate immunity of the lung is compromised, leading to repeated bacterial infections. This defect has been claimed to depend on inactivation of human β-defensin-1, which is inactive at high NaCl concentrations (33). Our data suggest that changes in the microenvironment, such as e.g. those encountered in cystic fibrosis lung, could influence the conformation of antibacterial peptides and thus modulate their activities.

To determine if peptide length influences the conformation and/or biological activity, the two N-terminally truncated variants FF-33 and SK-29 were synthesized. In 5 mM Na$_2$SO$_4$ LL-37 has considerable helicity, while FF-33 is significantly less helical (Fig. 6A). Accordingly, LL-37 is more active against *E. coli* D21 than FF-33 at this salt concentration (Fig. 6B). The antibacterial activity of FF-33 in medium E, however, is similar to that of LL-37 (Fig. 6C), showing that the four N-terminal amino acids as such are not essential for the antibacterial activity. Recently, a mouse cathelicidin, murine cathelin-like protein, was identified (34), where the C-terminal 37 residues have 43% sequence identity with LL-37, indicating that murine cathelin-like protein is the mouse homologue of human LL-37. A 29-residue putative antibacterial peptide derived from the C-terminal part of murine cathelin-like protein exhibited a disordered structure both in water and in medium E and had no antibacterial activity (34). In contrast, the corresponding human peptide SK-29 does adopt an α-helical secondary structure and is antibacterial in salt solutions. However, SK-29 requires higher concentrations of SO$_4^{2-}$ for induction of maximal helical content compared with LL-37 (Fig. 6A). In line with this result, the antibacterial activity of SK-29 against *E. coli* D21 is lower than that of LL-37 both in 5 mM Na$_2$SO$_4$ and medium E (Fig. 6, B and C).

Cytotoxic Activity of LL-37 and Serum Inhibition—Many antibacterial peptides (e.g. defensins (2), indolicidin (35), BMAP-27 (36), and LL-37) also exhibit cytotoxic effects against eukaryotic cells, but usually at higher concentrations compared with the bactericidal activity. The differences in effective bacterial and cytotoxic concentrations could lie in the different membrane compositions of eukaryotic and prokaryotic cells. However, the primary structures of BMAP-27 and BMAP-28 influence their membrane specificities, since the hydrophobic C-terminal tail of these peptides is needed for cytotoxic, but not antibacterial, effect (36). LL-37 lacks a hydrophobic tail, suggesting a different mechanism for its cytotoxicity. Cytotoxic effects may well be physiologically relevant at sites of inflammation, where antibacterial/cytotoxic peptides are induced in epithelial cells and/or recruited from granulocytes (5, 7). This could result in a high local concentration of peptide, leading to cytotoxicity. Examples of protective mechanisms against cytotoxic activities in the circulation do exist, where for example α2-macroglobulin works as a scavenger for the defensins (37). We have demonstrated that the antibacterial (Fig. 8) and cytotoxic activities of LL-37 are inhibited by human serum. Since we have no indication of degradation of LL-37 in human serum, it is possible that this inhibition reflects binding of LL-37 to a serum protein.

In conclusion, LL-37 requires an α-helical, oligomeric conformation for optimal antibacterial activity, and this conformation is dependent on LL-37 concentration and several factors in the microenvironment. Potentially harmful cytotoxic effects exerted by LL-37 against host cells are apparently attenuated by one or several factors in human serum.

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