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Structure-Function Studies of Antimicrobial and Endotoxin Neutralizing Peptides

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Introduction

In addition to the rapid emergence of multi-resistant bacteria, classical antibiotics may release endotoxin during the process of killing bacteria thus promoting endotoxic shock [1], claiming hundreds of thousands of lives yearly. To respond to the urgent need for antibiotics with novel mechanisms of action and for effective compounds to combat sepsis, we have designed antibiotic and anti-septic drug candidates by modification of a host defense peptide, a fragment of human lactoferrin (LF11, FQWQRNIRKVR-NH_2) with hydrophobic groups. A set of peptides was prepared by inserting hydrophobic groups varying in length and nature of their chains at either terminus. The antimicrobial activities of peptides against a panel of strains and their cellular toxicity were compared. Information on the peptide conformation in solution and upon interaction with membrane mimetic environments and LPS, and the effects the peptides may have on membrane mimetic systems were also investigated.

Results and Discussion

LF11 belongs to an amphipathic α-helical region of lactoferrin that is distinct from the site of iron binding and that represents part of the LPS binding region (residues 28-34) [2]. As shown by NMR, LF11 forms a well-defined hydrophobic core, when bound to LPS, allowing the basic residues to interact with the phosphate groups of LPS, the side chain of Phe-1 close to the aliphatic chains of lipid A, and Trp-3 at the interface between polar and nonpolar groups of the lipid A. The structure in anionic micelles forms a loop, which contains a kink at the N-terminus, positioning the hydrophobic chains towards the nonpolar interior of the micelles, and the basic groups spread around at the polar surface, interacting with anionic headgroups.

Derivatization of LF11-homoserine lactone derivative (LF12) with alkylamines of varying hydrocarbon chain lengths showed that the addition of a C12 alkyl chain to LF12 lead to the largest increase in antimicrobial potency, LPS binding and neutralizing activity compared to LF11 [3]. Further studies were then performed on a derivative of LF11 having a lauryl group at the N-terminus (C12-LF11). Neither LF11 nor C12-LF11 exhibited toxicity toward HeLa cells at 500 μg/ml (MTT assay) or hemolytic activity against human red blood cells at 100 μg/ml. As compared to LF11, C12-LF11 shows increased antimicrobial activity against several Gram-negative and Gram-positive bacteria determined by the microdilution method in Mueller-Hinton broth according to the NCCLS guidelines (Table 1).

The interactions of LF11 and C12-LF11 with LPS were analyzed using the deep rough mutant LPS from Salmonella minnesota (R595). Both peptides were able to neutralize the LPS induced production of TNFα by mononuclear cells as well as macrophages. The peptide/LPS affinity was rather similar for both peptides, as

Table 1. Antimicrobial activity of peptides

Bacteria	Minimum Inhibitory Concentration (μg/ml)[a]		
	LF11	**C12-LF11**	**Polymyxin B**
Escherichia coli (ATCC 25922)	256	64	0.5
Acinetobacter baumanii (10817/01 CUN)	>256	64	0.25
Bordetella bronquiseptica (10844/99 CUN)	128	8	0.125
Neisseria meningitides (10827/01 CUN)	>256	64	16
Staphylococcus aureus (ATCC 25923)	>256	128	8
Streptococcus pyogenes (ATCC 19615)	>256	64	8

[a]*MIC is the minimum peptide conc. where bacterial growth was not observed at 18h*

evidenced by the displacement of ^{45}Ca from LPS monolayers (Fig. 1). However, C12-LF11 was much more efficient to compensate the surface charge of LPS aggregates, a prerequisite for LPS neutralization, indicating that C12-LF11 inserts into the hydrophobic core of LPS aggregates.

Microcalorimetry showed that LF11 only negligibly affects the phase behavior of negatively charged dipalmitoyl-PG and zwitterionic dipalmitoyl-PE model membranes, while C12-LF11 strongly decreases the main transition temperature and the cooperativity of both lipids, and abolishes the pretransition around 33°C, as confirmed by wide-angle X-ray diffraction experiments. While LF11 only binds to the lipid surface, C12-LF11 inserts into the hydrophobic core of the membrane being consistent with its enhanced antimicrobial activity.

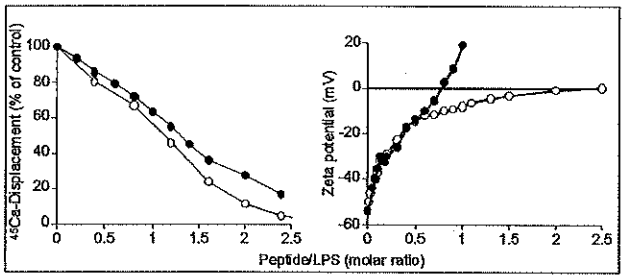

Fig. 1. Displacement of 45Ca2+ from LPS RE monolayers (left) and surface charge compensation of LPS RE aggregates (right) by LF11 (○) and C12-LF11 (●)

Acknowledgments

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