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The Acyl Group as the Central Element of the Structural Organization of Antimicrobial Lipopeptide

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Incorporation of non-natural amino acids or non-amino acid groups into peptides, such as β-amino acids, carbohydrates, nucleotides, or fatty acids extends their spectrum of activities as well as the accessible conformational space. In most of the determined structures of such hybrids, the peptide and non-peptide segment are segregated into two separate domains. Here we report on the solution structure of an antimicrobial lipopeptide, which shows that the acyl chain is tightly intertwined with the amino-acid residues. An acyl chain is present on several natural peptide antibiotics produced by nonribosomal synthesis. The acyl chain of polymyxin B, the archetypal lipopolysaccharide (LPS) neutralizing lipopeptide is essential for its antimicrobial activity.[1] Following the paradigm of polymyxin B, conjugation of cationic antimicrobial peptides with fatty acid chains improved their antimicrobial activity against Gram-negative and Gram-positive bacteria but also neutralization of LPS.[2−4] An ideal antimicrobial agent should display not only antimicrobial activity but also the ability to bind to and neutralize bacterial endotoxin in order to prevent septic (or endotoxic) shock, which accounts for more than 200 000 deaths in the U.S. every year.[5]

We have shown previously that the lipid environment significantly affects the conformation of LF11 (FQWQRNIRKVR-NH_2), a short antimicrobial peptide derived from human lactoferrin.[6] The acyl chain has been generally treated as a simple hydrophobic "appendix" that exerts no significant structural effects in the peptide segment,[7−9] and the improvement of antimicrobial and endotoxin-neutralizing activities has been attributed to a simple increase of bulk hydrophobicity.[2,10−15] In this paper we report on the effect of N-terminal acylation of this peptide (C12LF11 or *N*-lauryl-LF11, CH_3-$(CH_2)_{10}$-CO-NH-FQWQRNIRKVR-NH_2) on the structural organization of the peptide in the membrane mimetic environment, which is reflected in its antimicrobial activity modulated also by bacterial LPS. In order to discern the role of LPS on the antimicrobial activity of C12LF11, we used a collection of six isogenic *Salmonella minnesota* strains carrying well-defined mutations in genes involved in the biosynthesis of LPS. These mutants make rough LPS differing in their carbohydrate core size. Thereby the antimicrobial activity of C12LF11 was inversely related to the carbohydrate core size. Further, in all strains the lipopeptide activity was 4−16 fold better than the nonacylated peptide (Table S2). This is in agreement with the proposed effect of the negatively charged carbohydrate groups of LPS, which can act as a "sponge" to prevent binding of cationic peptides to the membrane inserted lipid A group.[16] The C12LF11 peptide also significantly decreased the mortality of mice inoculated with *P. aeruginosa* LPS (to be described elsewhere). The increased affinity of the lipopeptide for LPS results in the elimination of the transferred NOE effect that would allow structure determination of the complex as previously for the parent peptide LF11,[6] but may facilitate the transport across the outer membrane as the self-promoted cell uptake of polymyxin B.[17] Nevertheless, CD spectra show that LPS has a larger structuring effect on C12LF11 than on the nonacylated peptide (Figure S3, Supporting Information). Furthermore, in aqueous environment no persistent structure of C12LF11 was observed, while a significant effect of acylation on the peptide's conformation in the membrane-mimetic environment is seen, which is more prominent in DPC than in SDS (Figure S3).

Structure of C12LF11 in Dodecylphosphocholine (DPC) Micelles. 2D NOESY NMR spectra of C12LF11 in the presence of DPC (single chain phosphocholine) micelles revealed a large number of cross-peaks that could be unanimously assigned because of the high dispersion of the signals, notably including those between the peptide and the acyl chain (Figure 1).

The hydrogen atoms attached to the α, β, and γ C atoms of the acyl chain showed NOEs with Phe^1, Gln^2, Trp^3, and Gln^4. The refined C12LF11 structure in DPC micelles was well-defined with a backbone rmsd of 0.44 Å for residues 2−10 (Figure S1). It contained two short α-helical stretches (Phe^1, to Arg^5 and Asn^6 to Arg^{11}) tilted at an angle of 90°. Arg^5, Arg^8, Lys^9, and Arg^{11} formed an almost linear cluster of positively charged residues, whereas the acyl chain atoms, Phe^1, Gln^2, Trp^3, Ile^7, and Val^{10} were grouped in a hydrophobic patch, which gave the molecule a distinct amphipathic character.

Structure of C12LF11 in Sodium Dodecylsulphate (SDS) Micelles. The α and β protons in the acyl chain showed NOEs with Phe^1-H^δ and aromatic protons of Trp^3. The refined C12LF11 structure in SDS was less defined than in zwitterionic DPC (backbone rmsd for residues 2−10 1.72 Å (Figure S2)) in agreement with CD data. The residues Asn^6, Ile^7, Arg^8, Lys^9, and Val^{10} were organized in an extended conformation, with the side chains of the four basic residues Arg^5, Arg^8, Lys^9, and Arg^{11} forming a concavely shaped surface. The hydrophobic core (Phe^1, Trp^3, Ile^7, and Val^{10}) was significantly better defined (heavy atom rmsd 1.64 ± 0.71 Å) than the rest of the peptide; its side chains were oriented opposite

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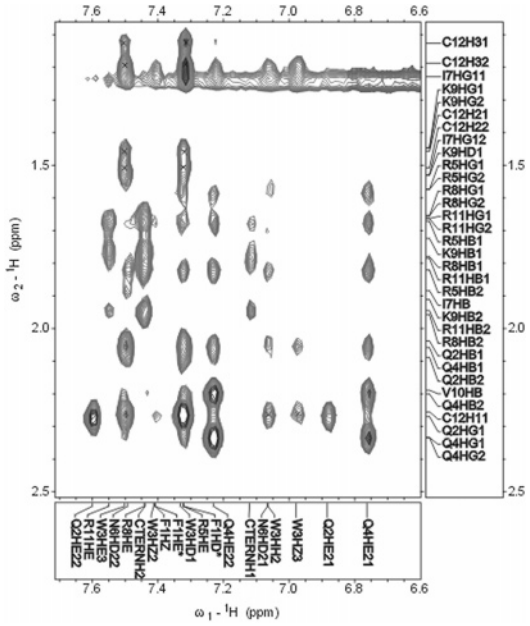

Figure 1. Section of the NOESY NMR spectrum indicating NOEs between the acyl chain and side-chain protons of Phe1 or Trp3 (crosses) demonstrating that the acyl chain of C12LF11 lipopeptide is well defined.

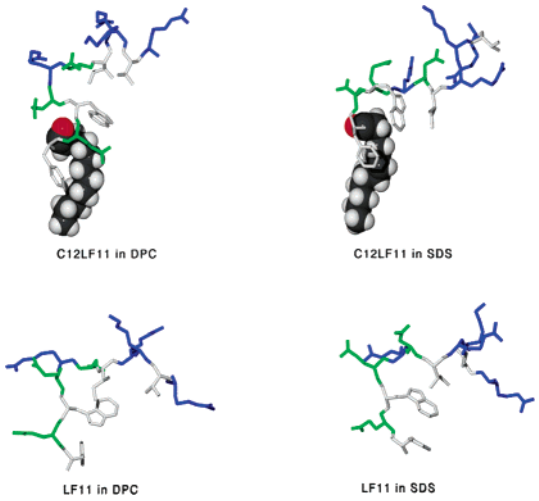

Figure 2. Comparison of the representative conformations of C12LF11 and LF11 in SDS and DPC. The acyl chain significantly changes the peptide fold and is in the lipopeptide tightly intertwined with the hydrophobic N-terminal residues. The *N*-lauryl chain of C12LF11 is displayed as a space-filled model.

to the positively charged surface. Gln[2] is only slightly shifted to the low field, suggesting that in contrast to DPC in SDS, it is not close to the aromatic rings of Phe[1] and Trp[3]. Nevertheless, the *N*-lauryl chain of the lipopeptide directly interacts with several N-terminal hydrophobic side chains in both types of micelles. Owing to the attraction of hydrophobic side chains, its effect, however, is propagated much further, determining the fold of the peptide.

In the micellar environment the parent peptide LF11 folds into a loop with a hydrophobic core organized around the Trp[3] residue to which Phe[1], Ile[7], and Val[10] are connected (Figure 2).[6] In the lipopeptide the acyl chain assumes the role of tryptophan as the "pivot" of the hydrophobic core, interacting extensively with both Phe[1] and Trp[3] residues, thus hindering their direct interaction. Contrary to the parent peptide LF11, the structure of C12LF11 is better defined in DPC than in SDS micelles as shown both by NMR and CD data. This is also reflected in the membrane mimetic study showing that the lipopeptide inserts into both zwitterionic and anionic membranes, thereby perturbing the lipid-chain packing and resulting in the decreased selectivity between bacterial and eukaryotic cells.[13] An extended acyl chain of the lipopeptide, which could fill the space in the hydrophobic core of the membrane would be primarily expected to increase the peptides' affinity for the membrane but not to increase the bilayer defects. However, in our case the *N*-lauryl chain interacts with the peptide residues creating packing defects in the hydrophobic core of the membrane, which cause the increased trans-gauche isomerization in the membrane lipid chains.

In most of the determined structures of lipopeptides the host peptide formed a defined secondary (predominantly α-helical) structure[7,8,10,15] or was stabilized either by the cyclization[18,19] or by a bound cofactor.[19,20] Linear peptides with no regular secondary structure are therefore expected to be much more sensitive to the effect of an acyl chain than longer peptides which are already "locked into" a secondary structure. Particularly in short amphipathic peptides the structural domination of the acyl chain over the aromatic residues is probably due to its larger nonpolar surface area. Despite its location at the N-terminus it forces the polypeptide chain to wrap around the acyl chain. The described structural effects of acylation demonstrate that it represents a handle to alter the peptide conformation and improve its bioactivity.

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Supporting Information Available: Detailed description of methods; Table S1 with structure statistics; Table S2 with antimicrobial activity; Figures S1−S3 with the NMR structure ensembles of C12LF11 in DCP and SDS, and CD spectra of C12LF11, also in mixture with LPS, DCP, and SDS. This material is available free of charge via the Internet at http://pubs.acs.org.

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