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Determination Of The Mechanism Of Action Of Peptides With Antimicrobial Potential By ^{31}P And ^{2}H Solid-state NMR

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A wide variety of organisms produce antimicrobial peptides as part of their first line of defense. These short cationic peptides are being considered as a new generation of antibiotics and represent great hopes against multiresistant-resistant bacteria which are an important clinical problem. Despite their diversity, the main target of antimicrobial peptides is the membrane(s) of pathogens. Previous studies have shown that a non-natural peptide composed of 14 residues (10 leucines and 4 phenylalanines modified with a crown ether) is able to disrupt negatively charged lipid bilayers. This peptide, called 14-mer, is of particular interest to lyse bacterial membranes. Biophysical studies suggested that the peptide binds to the membrane surface and induces pores stabilized by the peptide inverse-cone shape. However, the 14-mer is also able to disrupt neutral bilayers, limiting its application as antibiotic. To gain specificity against negatively charged membranes, several leucines have been substituted by positively charged residues (lysine, arginine, histidine).

Solid-state NMR experiments performed in model membranes were used to better characterize the mode of action of the charged peptides. More specifically, ^{31}P NMR provided information about the phospholipid polar head group, while ^{2}H NMR was used to measure the effect on the lipid acyl chains. Results obtained by a combination of ^{2}H, ^{31}P and ^{15}N NMR spectroscopy suggest that the peptides arrange themselves preferentially near the bilayer interface perturbing the membrane by the formation of pores. Lipid bilayers oriented between glass-plates were used to verify this hypothesis, while REDOR NMR experiments will be used to determine specifically which type of helical conformation is favored by these peptides.

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Biophysical Characterization And Membrane Interactions Of Peptides With Antimicrobial Potential

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It has been estimated that multiresistant bacteria present in hospitals are responsible for 2.5 millions of infections and of several thousands deaths each year in North America. The development of new classes of antibiotics is thus very important to fight against these bacteria. Amphipathic peptides with cationic charges represent one of these new classes. These peptides act by disrupting negatively charged bacterial membranes and have less effect on neutral eukaryotic plasma membrane.

We have previously shown that a non-natural peptide composed of 14 non charged residues (10 leucines and 4 phenylalanines modified with a crown ether) is able to disrupt bilayers but without selectivity (1, 2).

To gain specificity against negatively charged membranes, several leucines of this 14-mer have been substituted by positively charged residues (lysine, arginine, histidine). Biological tests indicate that some peptides are active against E. coli but ineffective against human red cells. These compounds have thus interesting properties to be use as antibiotics in the future.

In our group, we study these peptides by biophysical methods in order to better understand their mode of action on membranes. Fluorescence and Fourier transform infrared spectroscopies studies indicate that selective peptides disrupt negatively charged membranes but have no effect on neutral membranes. These methods, as well as dynamic light scattering and solid-state NMR also suggest that the peptides induce pore formation in the target membranes. This ability is related to the ability of peptides to be mainly in alpha-helix structure.

References :
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Can Peptide-Lipid Interactions Predict Bactericidal and Hemolytic Activity in Antimicrobial Peptides?

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It is relatively simple to design highly amphipathic linear cationic beta-sheet peptides containing 10-to-11 amino acids that possess potent antimicrobial activity. Often, however, these peptides also are quite hemolytic, so that there is insufficient selectivity between bacterial and human cells. Peptides with little or no hemolytic (or other toxic) activity toward human cells at 100 or more times the minimum inhibitory concentrations toward bacterial cells might be potential candidates for clinical use as antimicrobials. Since these peptides typically exert their bactericidal action through membrane disruption, we are interested in how they interact with model lipid vesicles. Here, we investigated how a group of peptides all containing a single tryptophan residue interact with large unilamellar vesicles (LUV) consisting of either anionic phosphatidylglycerol (PG), neutral phosphatidylcholine (PC), mimicking a mammalian plasma membrane surface, or a 2:1 mixture of phosphatidylethanolamine (PE) and PG, mimicking an E. coli plasma membrane surface. Lipid-peptide interactions are assessed by: (1) peptide conformation using circular dichroism; (2) proteolytic degradation; and (3) quenching of tryptophan fluorescence by aqueous acrylamide and membrane-bound 10-doxyl-nonadecane. By comparing results in the absence and presence of LUV, we assessed three sets of peptides with (a) high antimicrobial and high hemolytic activity, (b) low antimicrobial and low hemolytic activity, and (c) high antimicrobial and low hemolytic activity. Our results demonstrate that the ability of these peptides to interact with LUV of defined lipid composition in most cases correlates well with their activities in bacterial and human cells.

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Molecular Mechanism of pEM-2 Activity

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Interactions between a short synthetic antimicrobial peptide pEM-2 composed of 13 amino acid residues (KKWRWWLKALAKK) derived from C-terminus of myotoxin II of *Bothrops asper* and model membrane were investigated by Langmuir Blodgett (LB) and Atomic Force Microscopy (AFM). Peptide-induced surface area increase at constant pressure was studied for monolayers of zwitterionic DPPC, anionic DPPG phospholipids and E-coli extract. Increase in the transition state pressure for DPPG monolayer with increasing pEM-2 concentration and the corresponding AFM images show miscibility between the peptide and anionic lipid. It was found that incorporation of the peptide into DPPG monolayers is 2-3- orders of magnitude faster than into DPPC. The results indicate that electrostatic interactions play a significant role in the pEM-2-membrane interactions.

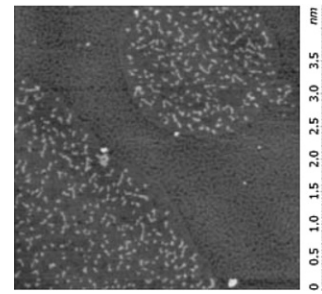

Figure 1. Contact mode AFM image (2x2 um) of a DPPG monolayer deposited at 30 mN/m in the presence of 400nM of pEM-2.

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Deamidation Weakens Membrane Binding Properties of Antimicrobial Peptide Anoplin

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Anoplin, GLLKRIKTLL-NH2, isolated from the venom sac of solitary spider wasp, *Anoplius samariensis,* is the smallest linear α-helical antimicrobial peptide found naturally up to date. Previously *Cabrera et al.* (J. Pept. Sci. 2008) reported that deamidation dramatically decreased antimicrobial activity of the peptide and showed that amidated Anoplin forms pores in toroidal manner in anionic bilayer. In the present work, interactions of two forms of Anoplin (Anoplin-NH2 and Anoplin-COOH) with model cell membrane (zwitterionic DPPC, anionic DPPG or *E. coli* extract) were further investigated in order to gain a better understanding of the effect of amidations on the kinetics and thermodynamics of the peptide- membrane interactions. Langmuir Blodgett, Atomic Force Microscopy, UV resonance Raman spectroscopy and Calcein leakage assay were used. Results of the study indicate that amidated form of Anoplin has higher membrane binding activity.

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The Activity of the Amphipathic Peptide delta-Lysin Correlates with Phospholipid Acyl Chain Structure and Bilayer Elastic Properties

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Release of lipid vesicle content induced by the amphipathic peptide delta-lysin was investigated as a function of lipid acyl chain length and degree of unsaturation for a series of phosphatidylcholines. Dye efflux and peptide binding were examined for three homologous lipid series: di-monounsaturated, di-polyunsaturated, and asymmetric phosphatidylcholines, with one saturated and one