Biophysical and antimicrobial investigations of D-PMAP23 affinity to bacterial membranes

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Antimicrobial peptides (AMPs) represent a newsworthy class of compounds endowed with expanding biological properties and a broad spectrum of action, making them a promising alternative for the development of new antibiotic drugs with a membrane-perturbing activity as primary mode of action.

Currently, the design of novel AMPs with improved activity, selectivity and resistance to degradation is a primary aim of research studies on therapeutic peptides. Biophysical experiments allow the quantitative determination of peptide-membrane association and the detailed characterization of the mechanisms of pore formation, but usually focus on model systems. Microbiological studies determine the total amount of peptide needed for bacterial killing, but the fraction of cell-bound peptides in these experiments is unknown. Trying to fill the gap between the two classes of studies, we recently determined the amount of cell-bound peptide molecules needed for killing a bacterium. In the case of the mammalian AMP D-PMAP23 we found that a total coverage of the bacterium by peptide molecules is necessary to cause its death [1]. With the goal of better characterizing the site of association of this large amount of peptide molecules, in the present work we performed binding studies with different bacterial strains, and with different components of the bacterial cell.

To understand the relevance of lipopolysaccharide (LPS) in peptide-cell association, we tested the AMP against two *Escherichia coli* mutant strains, *E.coli* D21 and D21f2, lacking of O-antigen- and O-antigen plus saccharidic portion, respectively. Fluorescence and antimicrobial activity studies have revealed no significant differences in peptide activity against LPS-mutant strains compared to wild-type E. coli ATCC 25922. This finding is in agreement with the low affinity of D-PMAP23 for LPS.

In parallel, the peptide affinity for different cell membrane components was analyzed against *E.coli* mutant strains lacking anionic lipids phosphatidylglycerol (PG) and cardiolipin (CL). Surprisingly, the affinity of D-PMAP23 for these strains and for the wild type was similar, and the antimicrobial activity was even slightly higher against the mutant strains. Furthermore, zeta potential measurements performed at increasing peptide concentrations showed that the peptide has a remarkable ability in neutralizing the anionic character of the bacterial cell surface. However, there was no substantial difference between wild-type and mutant bacteria strains. We explain these finding with an ability of the mutant strains to replace PG and CL with other anionic lipids, such as PA and DAG, so that the total membrane charge is essentially unaffected by the mutations [2].

Finally, to verify the ability of D-PMAP23 to penetrate into the cell and to bind intracellular components, we carried out fluorescence experiments on bacterial lysates and on the two resulting fractions obtained after centrifugation of the lysed sample, i.e. bacterial membrane extract and intracellular components. The affinity for the membrane extract was comparable to the affinity for live bacteria, indicating that in intact bacteria the peptide binds essentially to the cell membranes. However, peptide binding to the lysate or to the intracellular components was much higher. These findings suggest that once the peptide form pores in the membranes and gets access to the cytosol, it will probably accumulate intracellularly.
These findings provide novel insights into the mechanism of bacterial killing by AMPs, and open several new questions for future investigations.

[1] D.Roversi et al., ACS Chem Biol. 9 (2014) 2003-7

[2] Mileykoyskaya et al., J Biol Chem. 284 (2009) 2990-3000