Membrane Active Peptides II

1444-Pos
Effects of a Membrane-Active Amphibian Antimicrobial Peptide on the Bacterial Proteome

Donatella Barra1, Ludovica Marcellini1,2, Maria Luisa Mangoni1
1University of La Sapienza, Rome, Italy, 2University of Tor Vergata, Rome, Italy.

Ribosomally synthesized antimicrobial peptides (AMPs) are conserved components of the innate immunity of all life forms and represent the most ancient and efficient weapon against microbial pathogens. The emergence of multidrug-resistant microbes has urgently required the discovery of new antibiotics with a new mode of action, and AMPs represent promising candidates. Intense research focusing on AMPs is currently directed to the elucidation of their mode(s) of action. Nevertheless, very little is known about their effects on intact bacteria. Here we report on Esculentin 1-18 [Esc(1-18)], a linear peptide covering the first 18 N-terminal residues of the full length amphibian peptide esculentin-1b. Esc(1-18) retains the antimicrobial activity of esculentin-1b against a wide range of microorganisms, with negligible effects on mammalian erythrocytes. To expand our knowledge on the molecular mechanism underlying the antimicrobial activity on Gram-negative bacteria, we investigated the effects of this peptide on Escherichia coli, by studying its: i) structure in membrane mimicking environment; ii) killing kinetic; iii) bactericidal activity in different media; iv) ability to capillarize both artificial and bacterial membranes; v) capacity to synergize with conventional antibiotics; vi) effect on cell morphology and proteome by means of electron microscopy and proteomic techniques, respectively. These studies have indicated that Esc(1-18) (i) kills E. coli via membrane-perturbation; (ii) elicits identical changes in the bacterium’s protein expression pattern, at both lethal and sub-lethal concentrations; and (iii) preserves antibacterial activity under conditions closer to those encountered in vivo. This is in contrast with many host defence peptides that kill microorganisms by altering intracellular processes and lose activity in physiological systems. Importantly, to the best of our knowledge, this is the first case showing the effects of an amphibian AMP on the protein expression profile of its bacterial target.

1445-Pos
A New Look at an Old Friend: Novel Insights Into Pore Formation by Alamethicin

Aram J. Krauson, William C. Winley
Tulane University, New Orleans, LA, USA.

Alamethicin is a 20-amino acid long antibiotic peptide produced by the fungus Trichoderma viride. In the literature, alamethicin is the most commonly cited example of a barrel-stave model pore-forming peptide. In this model, amphipathic peptides form a long-lived transmembrane pore by aligning hydrophobic and hydrophilic residues with the lipid bilayer and aqueous pore respectively. It has been reported that voltage-independence of alamethicin pores relies on salt and peptide concentrations. We have developed a set of fluorescence-based assays for leakage, stable pore formation and lipid flip-flop using large unilamellar vesicles (LUVs) to help define the mechanism and potency of pore forming peptides. An additional pre-incubation assay differentiates dynamic pores from long-lived pores. When applied to alamethicin, our suite of assays show that, at 2.0 μM peptide, alamethicin forms voltage-independent pores in anionic or zwitterionic vesicles at peptide-to-lipid ratios as low as 1:2000. Less than 20 peptides per vesicle is sufficient to allow for complete vesicle permeabilization. Even after overnight incubation with vesicles, alamethicin promotes continuous lipid flip-flop, and is able to permeabilize multiple additions of new vesicles. Other pore forming peptides tested at this P:L ratio do not promote continuous flip-flop and do not exchange into new vesicles. We postulate that, unlike other pore-forming peptides, which mostly behave like classical barrel-stave pores, alamethicin is in a continuous dynamic equilibrium between transmembrane, interfacially bound and aqueous forms.
membrane. These results suggest that D1-7 eliminates the lytic activity but retains the strong lipid membrane binding. For further confirmation, we measured liposome sizes and zeta potentials with and without D1-7 loading. Consistently, D1-7 did not affect the size of the liposome, but shifted the zeta potential (or surface charge) of the liposome towards the positive voltage range, because D1-7 is a positively charged peptide. Among numerous existing nanosystems for drug delivery, liposomes are approved by FDA for anti-cancer and gene therapy. Accordingly, this linker and/or its refinements could enhance the therapeutic potential of approved liposomal drugs by enabling flexible incorporation and cargo multiplexing through post-formulation surface editing.

1449-Pos
Cationic, Helical Antimicrobial Peptoids with Biomimetic Antimicrobial Activity
Rinki Kapoor
Stanford University, Stanford, CA, USA.

Increasingly prevalent resistance of pathogenic bacteria to conventional small-molecule antibiotic drugs is creating an urgent need for the discovery of new classes of antibiotics that are active against biofilms. Bacteria that are multidrug resistant (MDR) are of increasing concern for infectious disease. Current treatment of these infections that involve resistant organisms may require 6-12 months of antibiotic treatment, creating difficulties with compliance. We are continuing to develop oligo-N-substituted glycine (peptoid) mimics of cationic, helical antimicrobial peptides (AMPs), and some of our recently acquired data indicate that peptoids could address the problem of growing resistance. Peptoids have been shown to have extremely broad-spectrum activity, and certain peptoids function well in the presence of serum proteins. Their biophysical mechanism of action makes it difficult for bacteria to evolve resistance to them. We have tested our most promising peptoids, peptides and commercial antibiotics in vitro against bacterial biofilms of a variety of important bacterial organisms. We show that certain peptoids can be as active as the preferred conventional antibiotics against bacterial infections, even at low micromolar doses. Small, structured biomimetic oligomers such as our antimicrobial peptoids may offer a new class of drugs that are useful in treating persistent bacterial infections.

1450-Pos
Investigation of a Sequence-Modified Antimicrobial Peptide
Luba Arotsky, Michael Urban, Gregory A. Caputo.
Rowan University, Glassboro, NJ, USA.

Antimicrobial peptides serve as one of the first lines of defense in the immune systems of higher organisms. These peptides specifically target and neutralize infecting bacteria in the host organism while exhibiting little or no toxic effect on host cells. The peptide C18G is a highly cationic, amphiphilic peptide derived from the C-terminal sequence of the human protein platelet factor 4 (involved in blood coagulation and wound repair) and exhibited antibacterial activity against both gram-positive and gram-negative bacteria. Using a modified C18G sequence that did not significantly affect antimicrobial efficacy (Tryr changed to Trp and all Lys changed to Arg (C18G Y3W K R)). The binding affinity was measured with fluorescence spectroscopy using the W in the peptide sequence as a probe of peptide environment. Small unilamellar lipid vesicles were used to investigate the binding affinity of the peptide to bilayers composed of variable amounts of DOPC, POPG, and POPE. DOPC and POPE have a zwitterionic head group, whereas POPG has an anionic charged head group. These studies showed binding affinity had a dramatic dependence on lipid composition. The effect of pH on peptide binding and behavior was also examined and, as expected, also impacted binding affinity. Quenching of the Trp fluorescence by acrylamide was performed to confirm that the Trp was located in the membrane. Likewise circular dichroism (CD) spectroscopy was used to determine the structure of the peptide upon interaction with the lipid vesicles. Additionally, in an assay monitoring membrane permeabilization of E. coli the C18G Y3W K R peptide was shown to permeabilize bacterial membranes in a concentration dependent manner.

1451-Pos
Structural Aspects of the Interaction of Nk-2 Derived Peptides with Cancer Cells
Yasemin Manavbasi1, Yana Gofman2, Nir Ben-Tal2, Regine Willumeit3, David Gidalevitz1,2.
1Yale University, New Haven, CT, USA, 2University of Rhode Island, Kingston, RI, USA, 3Technion-Israel Institute of Technology, Haifa, Israel.

Recently, antimicrobial peptides (AMPs) have emerged as a promising anticancer remedy. Negative charge of the bacterial membranes gives some measure for selectivity of cationic AMPs, since mammalian cell membranes are largely zwitterionic. Accumulating evidence indicates that lipid composition of the cancer cell membranes is different from a healthy cell, displaying net membrane surface negative charge. Understanding the nature of the negatively charged membrane domains could provide a new basis for anticancer therapy drug design using antimicrobial peptides or their synthetic mimetics. Here, we examine the effect of membrane glycosylation, which is shown to be increased in cancer cells, on activity of AMP analogs. In this work we probe interactions of antimicrobial peptide mimic, based on acyl-lysine architecture (OAK), C12 K-7αs, with Langmuir monolayers containing monosialoganglioside GM3 and disialoganglioside GD3. Langmuir isotherms and fluorescence microscopy imaging results of pure GM3 and GD3 monolayers indicate a single liquid-extended (LE) phase. Constant pressure insertion assays show significant insertion of C12 K-7αs in both GM3 and GD3 monolayers at 30 nM. AMP insertion inhibition was also observed for GM3; DPPC (30-70) and GD3 (30-70) mixed monolayers, however at smaller extent as expected. Synchrotron grazing Incidence X-Ray diffraction (GIXD) data show a disordered phase for GD3 and a weak ordering for GM3, which disappears immediately after introduction of the AMP. X-Ray Reflectivity data indicate the thinning of the lipid layer upon peptide insertion.

1453-Pos
Cancer Cell Proliferation is Inhibited by phiHLIP Mediated Delivery of Membrane Impermeable Toxin Phalloidin
Ming An1, Dayanjali Wijesinghe1, Oleg A. Andreev2, Yana K. Reshetnyak3, Donald M. Engelman1.
1Yale University, New Haven, CT, USA, 2University of Rhode Island, Kingston, RI, USA, 3University of North Carolina, Chapel Hill, NC, USA.

We wish to use the pH-(Low)-Insertion-Peptide (phiHLIP) to transport therapeutic agents to acidic tumors, with the ultimate goal of improving the treatment of cancer. phiHLIP inserts into a lipid bilayer under slightly acidic conditions (pH 6-6.5), forming a transmembrane helix. We demonstrate here that phiHLIP-mediated delivery of a cell-impermeable, polar toxin phallolidin can inhibit the proliferation of cancer cells. The delivery constructs, phiHLIP-Krho(C)aph and phiHLIP-C(aph), both carry the phallolidin toxin at the inserting C-terminus, via a disulfide linkage that could be cleaved in cells. The constructs differ in that a lipophilic rhodamine moiety is also attached to the inserting end, near the phallolidin cargo, in phiHLIP-K(rho)C(aph). After a brief incubation with 2-4 µM of phiHLIP-Krho(C)aph at pH 6.1-6.2 (for 1-3 h), proliferation of HeLa, JC, and M4A4 cancer cells were severely disrupted (> 90% inhibitions). Cells