4th International Meeting on Antimicrobial Peptides

September 29-30, 2014
Graz, Austria

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PROGRAM

MONDAY, September 29, 2014

8^{00} - 9^{00}	Registration and Mounting of Posters	
9^{00} – 9^{15}	**Welcome Address**	**Kai-Uwe Fröhlich,** Head of Institute of Molecular Biosciences, University of Graz **Harald Mangge,** Coordinator BioTechMed, Medical University of Graz, Austria
Session 1	**Model systems and mechanistic studies** **Chair: Georg Pabst and Kai Hilpert**	
9^{15} – 9^{50}	Plenary Lecture 1	**Erwin London,** Stony Brook University, New York, US Novel model membrane systems for defining lipid-peptide interaction
9^{50} – 10^{10}	Short Talk 1	**Robert Vacha,** Masaryk University, Brno, CZ Double-belt, a novel structure of a membrane pore
10^{10} – 10^{30}	Short Talk 2	**Alexander Jilek,** University of Vienna, A aDrs, an anionic peptide from frog skin
10^{30} – 11^{00}	**Coffee & Tea**	
11^{00} – 11^{35}	Plenary Lecture 2	**Thomas Gutsmann,** Research Center Borstel, D Interaction between host defence peptides and mycobacteria
10^{35} – 12^{10}	Plenary Lecture 3	**William F. Walkenhorst,** Loyola University, New Orleans, US The effect of pH, ionic strength, and specific ions on AMP activity
12^{10} – 12^{30}	Short Talk 3	**Nermina Malanovic,** University of Graz, A Interaction of OP-145, a derivative of human cathelicidin LL-37, with bacterial plasma membranes and cell wall components: impact of secondary structure and aggregation state
12^{30} – 13^{00}	**Lunch**	
13^{00} – 14^{30}		**Poster Session**

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Session 1 continued:

Session 1		Model systems and mechanistic studies Chair: Havard Jenssen and Dagmar Zweytick
$14^{30} – 15^{05}$	**Plenary Lecture 4**	**Burkhard Bechinger,** University of Strasbourg, F On the membrane interactions of antimicrobial peptides
$15^{05} – 15^{40}$	Plenary Lecture 5	**Ayyalusamy Ramamoorthy,** University of Michigan, Ann Arbor, US Atomic view of oligomerization, membrane disruption and toxicity by antimicrobial peptides and amyloid peptides
$15^{40} – 16^{00}$	Short Talk 4	**Niels Geudens,** Ghent University, B Membrane interactions of natural cyclic lipodepsipeptides
$16^{00} – 16^{30}$	**Coffee & Tea**	
$16^{30} – 17^{05}$	Plenary Lecture 6	**Marco Scocchi,** University of Trieste, I On the mode of action of the proline-rich peptide Bac7
$17^{05} – 17^{40}$	Plenary Lecture 7	**Gerard Wong,** University of California Los Angeles, US AMPs in infectious diseases and autoimmune diseases
$17^{40} – 18^{00}$	Short Talk 5	**Gabriela Eggiman,** Durham University, UK Developing new anti-infective peptide-mimetics
$18^{00} – 18^{20}$	Short Talk 6	**Vladimir Frecer,** Comenius University, Bratislava, SK Mechanistic quantitative structure-activity relationships and rational design of antimicrobial peptides

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TUESDAY, September 30, 2014

Session 2		Biofilms and implant coatings Chair: Sarah E. Maddocks and Regina Leber
$9^{00} – 9^{35}$	Plenary Lecture 8	**Sebastian Zaat,** Center of Infection and Immunity Amsterdam (CINIMA), Amsterdam, NL BALI beating biofilms
$9^{35} – 10^{10}$	Plenary Lecture 9	**Guillermo Martinez de Tejada,** University of Navarra, Pamplona, E Antimicrobial activity of synthetic peptides and lipopeptides derived from lactoferricin against *Pseudomonas aeruginosa* biofilms
$10^{10} – 10^{30}$	Short Talk 7	**Malgorzata Dawgul,** Medical University of Gdansk, P Influence of lipopeptides with two fatty acid chains on bacterial biofilm
$10^{30} – 11^{00}$	**Coffee & Tea**	
$11^{00} – 11^{35}$	Plenary Lecture 10	**Karin Thevissen,** Catholic University of Leuven, B Synergistic activity of peptides and common antifungal drugs on biofilms
$10^{35} – 11^{55}$	Short Talk 8	**Sona Kucharikova,** Catholic University of Leuven, B Novel class of peptides derived from a protein sequence of *Candida albicans* Als3 reduced attachment to plastic, mature biofilm development, adhesion and invasion to human epithelial cell lines
$11^{55} – 12^{15}$	Short Talk 9	**Yoo Jin Oh,** Johannes Kepler University Linz, A Characterizing the curli fimbriae on living bacterial surfaces using scanning probe microscopy and single molecular force spectroscopy
$12^{15} – 13^{00}$	**Lunch**	
$13^{00} – 14^{00}$		**Poster Session**

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Session 3		Towards application Chair: Karl Lohner
$14^{00} – 14^{35}$	Plenary Lecture 11	**Ralf Hoffmann,** University of Leipzig, D Insect-derived proline-rich antimicrobial peptides: Novel protein targets and transport mechanisms
$14^{35} – 14^{55}$	Short Talk 10	**Luzia Holfeld,** University of Leipzig, D Pharmacokinetics and tissue distribution of the proline-rich antimicrobial peptide Onc72
$14^{55} – 15^{15}$	Short Talk 11	**Anna Piras,** University of Pisa, I Chitosan nanoparticles for the linear release of cationic antimicrobial peptides
$15^{15} – 15^{35}$	Short Talk 12	**Edwin Veldhuizen,** Utrecht University, NL In ovo administration of the chicken cathelicidin peptide analog DCATH-2 protects against bacterial infection in young broilers
$15^{55} – 16^{10}$	Plenary Lecture 12	**Kai Hilpert,** St. George's University of London, GB Optimizing the therapeutic potential of short antimicrobial peptides
$16^{10} – 18^{00}$		**Fairwell Address with Coffee & Tea**

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Plenary Lectures

&

Short Talks:

Abstracts

Novel model membrane systems for defining lipid-peptide interaction

Erwin London

Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY, USA

The behaviors of membrane-inserted peptides and proteins are greatly influenced by membrane lipid composition. We previously developed fluorescence methods demonstrating that the configuration/topography of membrane-bound peptides is greatly influenced by lipid composition. This includes an effect of lipid composition upon both the extent that a membrane-associated hydrophobic peptide forms a transmembrane state and the positioning of the transmembrane state within the lipid bilayer. To allow investigation of lipid-peptide and lipid-protein interactions in more detail, we recently developed a lipid substitution procedure using cyclodextrins to carry out *in situ* substitution of one or more membrane phospholipids by others in an intact lipid vesicle. The method has been used to prepare asymmetric vesicles in which the lipid composition of the inner and outer leaflets differ. We find that lipid vesicles with asymmetry that mimics that in eukaryotic membranes can strongly influence the topography of membrane-inserted peptides and proteins relative to that in membranes that lack lipid asymmetry.

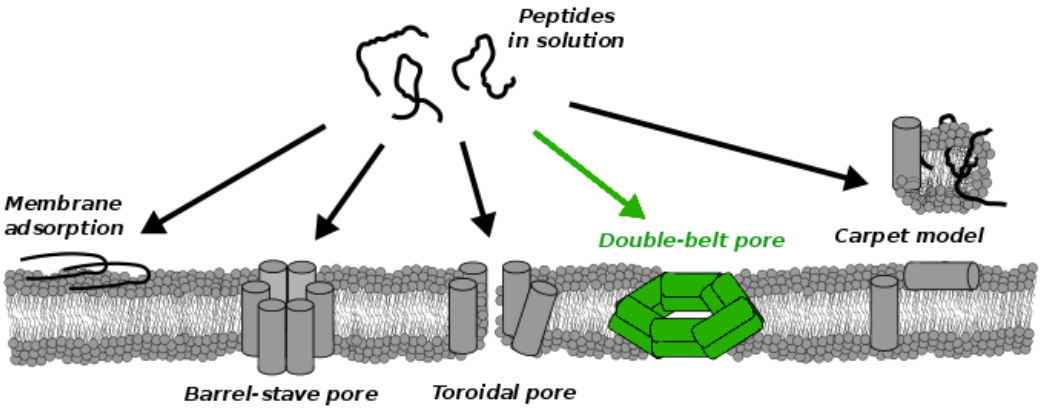
Notes:

Double-belt a Novel Structure of Membrane Pore

Robert Vacha[1], Daan Frenkel[2]

[1]Masaryk University, Brno, Czech Republic, [2]University of Cambridge, Cambridge, United Kingdom

Amphiphilic proteins and peptides can induce formation of stable and metastable pores in phospholipid membranes, which has been associated with toxicity or antimicrobial activity. Using coarse-grained simulations we have studied peptide orientation within the pores and have found that peptides can be oriented perpendicular, parallel, or tilted with respect to the membrane plane. The orientation depends on the length of the peptide and its hydrophobicity distribution, which we rationalized in terms of the hydrophobic mismatch. Apart from well-known barrel-stave or toroidal pores our simulations suggest a novel 'double-belt' pore structure, where peptides within the membrane pore are oriented parallel to the membrane plane. This result was verified using more detailed simulations with the MARTINI force field, where the double-belt structure was stable in micro-second time scale of our simulation.



Notes:

aDrs, an anionic peptide from frog skin

Gößler-Schöfberger R[1], Hesser G[2], Reif MM[3], Friedmann J[4], Toca-Herrera JL[4], Oostenbrink C[3], Jilek A[5].

[1]Institute of Organic Chemistry, [2]CSNA Center for Surface- and Nanoanalytics, Johannes Kepler University Linz, Austria, [3]Institute of Molecular Modeling and Simulation, [4]Department of NanoBiotechnology, University of Natural Resources and Life Sciences Vienna, Austria, [5]Institute of Biological Chemistry, University Vienna, Austria.

Anionic dermaseptin (aDrs), an unusual peptide from the skin secretions of the frog *Pachymedusa dacnicolor*, contains three aspartic acid residues resulting in a negative net charge at neutral pH, as opposed to numerous other dermaseptins which are helical cationic antimicrobial peptides (AMPs). Yet there is a similarity to cationic AMPs as the sequence can be fitted onto an amphipathic alpha helix by an Edmundson wheel projection. The peptide has an inherent propensity to self-assemble into amyloid fibrils in a pH-controlled fashion, which could play a functional role in defense. aDrs can be enzymatically converted into the diastereomer [D-Leu2]-aDrs by an L/D-isomerase. The morphology of fibrils formed by these isomers is controlled by the stereochemistry of residue 2, whereas kinetic and thermodynamic parameters of aggregation are barely affected.
Amyloid formation is reversible and above pH 5, the amyloid fibrils disassemble in a cooperative manner. At neutral pH, this process proceeds instantaneously to the soluble form. Within the transition interval (pH 5-6.5), however, 'backward' granular aggregates (10-500 nm) are formed. Such metastable amorphous aggregates, which are quickly released from an amyloid depot by a shift in pH, can mediate a strong cytotoxic effect *via* an as yet unidentified mechanism. Interestingly, the amorphous aggregates keep some structural characteristics of amyloid. It will be interesting to see in the future, whether this or related peptides represent a new class of AMPs with a distinct mode of action or whether they complement the action of CAMPs.

Notes:

Interaction between host defence peptides and mycobacteria

Christian Nehls[1], Max Koistinen[1], Sam Willcocks[2], Ulrich Schaible[1], and Thomas Gutsmann[1]

[1] Research Center Borstel, Germany
[2] London School of Hygiene and Tropical Medicine, Great Britain

Mycobacterium tuberculosis is resistant against the antimicrobial activity of a large number of host defence peptides (HDPs) in the phagosome, which are active against other Gram-negative or –positive bacteria. One reason for this resistance is the unique structure of the mycobacterial cell wall. Mycobacteria produce a thick mycolate-rich outer covering which functions as an exceptionally efficient barrier. We demonstrated that the membrane forming properties of trehalose dimycolate (TDM), as one of the important components of the mycobacterial outer barrier, are unique. TDM, which is organized in domains, leads to an increase of the overall membrane stability by a factor of three (as obtained in force spectroscopic experiments using atomic force microscopy).
Moreover, we used reconstituted planar lipid bilayers composed of TDM and phospholipids to perform electro-physiological experiments for determining the membrane permeabilizing activity of different HDPs. Furthermore, we used atomic force microscopy to characterize the surface topography of reconstituted membranes and mycobacteria. The biophysical results were compared with results from killing assays. Interestingly LL32, which is the highly active fragment of human cathelicidin, induced dramatic changes in the membrane morphology without inducing a significant permeabilization. LL32 is able to kill other Gram positive or negative bacteria by induction of membrane lesions, but up to a concentration of 30 µg/ml LL32 could not kill *M. tuberculosis*. Surprisingly, LL32 even stimulated an increased growth of the mycobacteria. In contrast two synthetic peptides based on LL32 and a linear version of the human beta-defensin-3 (hBD-3) showed a pronounced killing and permeabilization.

Notes:

The effect of pH, ionic strength, and specific ions on AMP activity

William F. Walkenhorst

Loyola University New Orleans, USA

We are investigating a family of cationic antimicrobial peptides (CAMPs) selected from a combinatorial library that exhibit potent, broad spectrum activity at neutral pH. The activity profiles of these peptides were probed by varying the effects of solution conditions such as pH and ionic strength on the performance of the peptide antimicrobials against a panel of microorganisms. Peptide minimum sterilizing concentrations (MSCs) varied linearly with pH for each subtype within our family of CAMPs for all organisms tested. The peptides were much less effective against Gram negative bacteria at high pH, consistent with a decrease in net positive charge on the peptides. A similar trend was observed for the fungus Candida albicans. Surprisingly, the opposite pH trend was observed with the Gram positive Staphylococcus aureus. The difference in the observed pH behavior is attributed to the presence of native charged molecules in the peptidoglycan layer of S. aureus. The novel species specific effects of pH observed here have important implications for applications using CAMPs and for the design of novel CAMPs. In addition, data will be shown for more recent work involving the combination of toxic and inhibitory ions with CAMPs to increase antimicrobial activity.

Notes:

Interaction of OP-145, a derivative of human cathelicidin LL-37, with bacterial plasma membrane and cell wall components: impact of secondary structure and aggregation status

N. Malanovic[1], R. Leber[1], M. Kriechbaum[2], J.W. Drijfhout[3] and K. Lohner[1]

[1] Institute of Molecular Biosciences, Biophysics Division, University of Graz, Austria,
[2] Institute of Inorganic Chemistry, Graz University of Technology, Austria
[3] Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, The Netherlands

OP-145, also known as P60.4Ac, a synthetic antimicrobial peptide derived from the human cathelicidin LL-37, has been successfully used as treatment of chronic otitis media before [1]. OP-145 displayed strong antimicrobial activity [2] but is also lytic to human cells at higher concentrations. Its mechanism(s) of action is (are) still unclear.
In the present study, we investigated the interactions between OP-145 and bacterial and mammalian model membranes to obtain better insight into peptide's specificity and its mode of action. Thermodynamic and structural studies using liposomes composed of phosphatidylcholine (PC) mimicking mammalian cell membranes showed that OP-145 induces disintegration of PC liposomes into disk-like micelles and bilayer sheets, suggesting a detergent-like action. The same studies using liposomes composed of phosphatidylglycerol (PG) mimicking Gram-positive bacterial membranes revealed formation of quasi-interdigitated lipid-peptide structures in the gel and membrane thinning in the fluid phase. Although, OP-145 interacted with both PG and PC systems, the extent of perturbation of bacterial cell membrane was shown to be higher. The presence of bacterial cell wall components lipoteichoic acid (LTA) and peptidoglycan (PGN) did not interfere with the activity of the peptide towards PG membranes. Indeed, OP-145 was capable of displacing LTA as well as PGN from DPPG membranes.
CD spectroscopy revealed α-helical structures of the peptide for both model systems, but the different oligomerisation grade of the peptide in these systems may explain the higher activity of OP-145 towards bacterial as against mammalian membranes.

References
[1] Peek et al. (L1-337). 2009. ICAAC.
[2] Nell et al. (2006) *Peptides* **27**, 649-660.
This work is supported by FP7 under grant agreement n° 278890 (BALI Consortium).

Notes:

On the membrane interactions of antimicrobial peptides

Evgeniy S. Salnikov, Christopher Aisenbrey, Arnaud Marquette, Elise Glattard, Burkhard Bechinger

Institut de chimie, UMR7177, University of Strasbourg / CNRS, 67000 Strasbourg, France

Biophysical investigations that characterize on a quantitative scale the interactions of cationic linear peptides with lipid bilayers will be presented. A focus our work is the use of solid-state NMR spectroscopy to investigate at the same time the structure, topology of the peptides as weel as the membrane phase behavior and curvature of the lipid bilayers. Such studies have changed our view how antimicrobial peptides work (1,2) and have resulted in the conceptually novel design of antibiotic compounds. In the following we aim to understand how some mixtures of these peptides exhibit synergistic activities. Therefore, the structure, topology and dynamics of PGLa and magainin 2, antimicrobial peptides from frogs, were investigated in oriented phospholipid bilayers using solid-state NMR in the presence or absence of the other peptide and as a function of the membrane lipid composition (2,3). Furthermore, fluorescence spectroscopy was used to investigate how the peptides interact with each other within the lipid bilayer environment.
Whereas, magainin 2 exhibits stable in-planar alignments under all conditions investigated PGLa adopts a number of different membrane topologies with considerable tilt angle variations (2,3). In bilayers, which represent closely the natural membrane composition (1-palmitoyl-2-oleoyl-phospholipids), both peptides adopt a surface oriented topology. These results have important consequences for the mechanistic models explaining synergistic activities of the peptide mixtures and will be discussed in the context of unpublished data where membrane structure and interactions are correlated with biological activities.

References:
(1) Bechinger, J. Pep. Scie, 17, 306-314 (2010)
(2) Bechinger, B., Resende, J., and Aisenbrey, Biophysical Chemistry 153, 115-125 (2011)
(3) Salnikov & Bechinger, Biophys J. 100, 1473-1480 (2011)

Notes:

Atomic view of oligomerization, membrane disruption and toxicity by antimicrobial peptides and amyloid peptides

Ayyalusamy Ramamoorthy, Jeffrey Brender, Samuel Kotler, and Dongkuk-Lee

Biophysics and Department of Chemistry, The University of Michigan, Ann Arbor

Membrane interaction and disruption by antimicrobial peptides and amyloid peptides are investigated using a variety of biophysical and biochemical approaches.[1-3] Using model lipid membranes (such as MLVs and LUVs, and aligned lipid bilayers and bicelles), we have investigated the aggregation and mechanism of action of several designed antimicrobial peptides (MSI-78 (commercially known as pexiganan), MSI-367, MSI-594 and MSI-843) and amyloid peptides such as amyloid-beta implicated in Alzheimer's disease and amylin involved in type-2 diabetes. The MSI peptides are capable of fragmenting LUVs through a carpet (detergent-type) mechanism depending on the amino acid sequence and/or the membrane composition.[1] The amyloid peptides function via a two-step process, with the initial formation of ion-selective pores followed by nonspecific fragmentation of the lipid membrane during amyloid fiber formation. Our results show that the presence of ganglioside enhances both the initial pore formation and the fiber-dependent membrane fragmentation process.[2,3] High-resolution solid-state NMR experiments are used to reveal the structure, oligomerization and function.

References:
1. *Solid State Nucl. Magn. Reson.* **35**, 201-207 (2009).
2. *Chem. Soc. Rev.* **43**, 6692-6700 (2014).
3. *Acc. Chem. Res.* **116**, 3650–3658 (2012).

Notes:

Membrane interactions of natural cyclic lipodepsipeptides

N. Geudens[1], K. Fehér[1], M. De Vleeschouwer[2], Jean-Marc Crowet[3], Mehmet Nail Nasir[3], A. Madder[2], Laurence Lins[3], J.C. Martins[1] and D. Sinnaeve[1]

[1] Ghent University, NMR and Structural Analysis Unit
[2] Ghent University, Organic and Biomimetic Chemistry
[3] University of Liege, Gembloux, Unité de Chimie Biologique Industrielle

Cyclic lipodepsipeptides (CLPs) are non-ribosomal peptides produced by bacteria, mainly *Pseudomonas* and *Bacillus* spp. They consist of a short sequence of both D- and L-amino acids which forms a cyclic structure by means of an ester bond between the C-terminus and a side-chain alcohol. They possess interesting antimicrobial activities.
In the past, efforts have been made in analysing the conformations and self-assembling properties of a collection of CLPs known as the viscosin group. [1-4] It is hypothesized that the antimicrobial activity of CLPs can be related to their ability to interact with cell membranes. We will present a multidisciplinary approach to better understanding of the working mechanism of the CLPs by focussing on the peptides and/or on the lipid bilayer itself.
NMR spectroscopy has emerged as a powerful tool for the structure determination of peptides in the presence of model membranes. In solution-state NMR, it is important that the model membrane is sufficiently small to obtain good spectral resolution. This requirement imposes a restriction on the types of model membranes that are suitable. In this respect, isotropic bicelles have emerged as model membranes for NMR that combine the advantageous NMR properties of micelles with the characteristics of lipid bilayers. [5]
Additionally, complementary biophysical experiments are performed, including fluorescence spectroscopy and circular dichroism. To better understand the molecular mechanism of CLPs at an atomic level, preliminary Molecular Dynamics simulations in the presence of bilayers will also be presented.

1. Sinnaeve, D., P. M. Hendrickx, *et al.* (2009) Chemistry - A Europ. Journal **15**(46): 12653-12662.
2. Sinnaeve, D., M.-A. Delsuc, *et al*. (2012) Chemical Science **3**: 1284-1292.
3. De Vleeschouwer, M., D. Sinnaeve, *et al. (2014)* Chemistry - A Europ. Journal **20**(25): 7766-7775
4. Geudens N., De Vleeschouwer M., *et al* (2014) ChemBioChem, *in press*
5. Durr, U. H., M. Gildenberg, *et al*. (2012) Chem Rev **112**(11): 6054-6074.

Notes:

On the mode of action of the proline-rich peptide Bac7

M. Mardirossian[1], G. Runti[1], P. Mergaert[2], C. Giglione[2], M. Benincasa[1], F. Guida[1], R. Gennaro[1], A. Tossi[1] and M. Scocchi[1]

[1] Department of Life Sciences, University of Trieste, Italy.
[2] Institut des Sciences du Végétal UPR 2355 CNRS, Gif-sur-Yvette, France.

Most Antimicrobial peptides (AMPs) inactivate bacteria through pore formation or membrane barrier disruption. Other AMPs, including the Proline-rich peptides (PR-AMPs), cross the bacterial membranes without significant damages and act inside the cells affecting vital processes. An important role in the internalization or PR-AMPs into Gram-negative bacteria is played by the inner membrane protein SbmA and the chaperone DnaK has been identified as a target for some of these peptides. Despite these insights the precise mode of action of PR-AMPs remains to be revealed. By using a 35-mer peptide derived from Bac7, a proline-rich AMP belonging to the cathelicidin family, we investigated the functional features of SbmA-mediated transport, the ability of the transporter to bind the peptide, and the concentration reached by Bac7 inside the *E. coli* cytoplasm. We evaluated its *effects* on the *in vitro* transcription and/or translation processes. Assays of incorporation of radioactive precursors of macromolecules with living *E. coli* cells were performed to confirm the results obtained *in vitro*. As translation was found to be affected by the peptide, ribosome-co-precipitation and crosslinking assays were performed looking for interactions between the peptide and ribosomal proteins.
The results of this study represent an advance in the understanding of the functioning of this type of antimicrobial peptides and represent a promising starting point for the design of new antibiotics.

Notes:

Infectious diseases, auto-immune diseases, and opportunities for bioengineering

Gerard C.L. Wong

Bioengineering Dept., Chemistry & Biochemistry Dept., California NanoSystems Institute, UCLA, USA

We present examples from our group where bioengineering and biophysics impact unsolved medical problems. We start with bacterial biofilms, which are structured multi-cellular communities that are fundamental to the biology and ecology of bacteria. The first step in biofilm formation, adaptation to life on a surface, requires the coordination of biochemical signaling, polysaccharide production, and molecular motility motors. These crucial early stages of biofilm formation are at present poorly understood. By adapting tracking algorithms from colloid science, we dissect bacterial social behavior at the single cell level. We will also discuss how we can learn from innate immunity peptides in antibiotic design, and the pathological role of such peptides in autoimmune diseases.

Notes:

Developing new anti-infective peptide-mimetics

Gabriela A. Eggimann,[1] Hannah L. Bolt,[1] Gary J. Sharples,[1,2] Paul W. Denny,[1,2] Steven L. Cobb[1]

1 Department of Chemistry, Durham University, UK
2 School of Biological and Biomedical Sciences, Durham University, UK

Antimicrobial peptides (AMPs) have been proposed as one potential solution to the development of new antibacterial and anti-parasitic drugs to cure skin infections e.g. acne or cutaneous leishmaniasis (CL).[1] However, their inherent chemical and biological instability presents a major hurdle and only a few AMPs are currently in clinical trials for the treatment of skin infections. Early progress with synthetic peptide mimetics suggests that these molecules offer a significantly better opportunity for development from bench to market. Amongst the AMP mimetics reported, peptoids have considerable potential for the development of new topical anti-infective agents.[2] Relative to AMPs, antimicrobial peptoids are cheaper to manufacture and have significant therapeutic potential as a consequence of their structural stability, superior bioactivity and resistance to protease degradation. They also retain broad spectrum activity against multidrug resistant bacterial strains, as shown in literature.[3] We have recently identified for the first time selected peptoids with anti-parasitic activity against *Leishmania mexicana* promastigotes and axenic amastigotes causing CL (submitted). These peptoids are active in the low µM range and were used as leading compounds in a structure-activity-relationship (SAR) study to elucidate the mode of action against the parasite and their toxicity against skin cells. In addition, we also screened our peptoid library against different strains of gram-positive and gram-negative bacteria to identify new compounds with high antibacterial activity in the low µg/mL range. Here we will present the work we have carried out to develop peptoids as an entirely new and promising class of anti-infectiv agents.

Key references:

[1] S. L. Cobb, P. W. Denny et al. *J Pept Sci.* **2011**, *17*, 751-755
[2] R. N. Zuckermann et al. *Curr. Opin. Mol. Ther.* **2009**, *11*, 299-307.
[3] A. E. Barron et al. *PNAS* **2008**, *105*, 2794-2799**.**

Notes:

Mechanistic Quantitative Structure-Activity Relationships and Rational Design of Antimicrobial Peptides

Vladimir Frecer [1], Jakub Kollar [1,2], Adam Hotra [1]

[1]Department of Physical Chemistry of Drugs, Faculty of Pharmacy, Comenius University, Bratislava SK-83232, Slovakia, [2] Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava SK-84215, Slovakia

Gram-negative bacteria release lipopolysaccharides (LPS) during infection and antimicrobial therapy, which may cause lethal endotoxic shock syndrome. Rational design strategy based on the presumed mechanism of antibacterial action of β-sheet cationic antimicrobial peptides on bacterial membrane was adopted to design antimicrobial peptides capable of binding LPS [1,2]. The designed peptides forming symmetric β-hairpin amphipathic structures displayed binding affinities to LPS and lipid A in the low micromolar range [2]. They also exhibited strong effects against gram-negative bacteria, with MICs in the nanomolar range, and low cytotoxic and hemolytic activities at concentrations significantly exceeding their MICs. Quantitative structure-activity relationships (QSAR) analysis of peptide sequences and their antimicrobial, cytotoxic and hemolytic activities revealed that site-directed substitutions of residues in the hydrophobic face of the amphipathic peptides with less lipophilic residues selectively decrease the hemolytic effect without significantly affecting the antimicrobial or cytotoxic activity. On the other hand, the antimicrobial effect can be enhanced by substitutions in the polar face with more polar residues, which increase the amphipathicity of the peptide. The findings highlight the importance of peptide amphipathicity and allow a rational method that can be used to dissociate the antimicrobial and hemolytic effects of cationic β-hairpin peptides.
This mechanistic QSAR model was applied to optimization of antimicrobial and hemolytic activities of porcine protegrin-1 (PG-1) mimetics synthesized by Robinson *et al.* [3]. These cyclic cationic peptides with β-hairpin fold were analyzed by the coarse-grain QSAR model, which uses additive molecular properties related to the mechanisms of bacterial cell membrane disruption that can be easily calculated from available data on amino acids [4].

References

1. Frecer, V., Ho, B., Ding, J. L.: *Eur. J. Biochem.* **267**(3), 837-852 (2000).
2. Frecer, V., Ho, B., Ding, J. L.: *Antimicrob. Agents Chemother.* **48**(9), 3349-3357 (2004).
3. Robinson, J. A., Shankaramma, S. C., Jetter, P., Kienzl, U., Schwendener, R. A., Vrijbloed, J. W., Obrecht, D.: *Bioorg. Med. Chem.* **13**(6), 2055-2064 (2005).
4. Frecer, V.: *Bioorg. Med. Chem.* **14**(17), 6065-6074 (2006).

Notes:

BALI beating biofilms

S.A.J. Zaat, on behalf of the BALI consortium (www.bali-consortium.eu)
Dept. of Medical Microbiology, Center of Infection and Immunity Amsterdam (CINIMA), Amsterdam, The Netherlands

Infection of inserted and implanted medical devices (biomaterials) is a major problem in modern healthcare. Staphylococci are the predominant cause of these infections. The pathogenesis involves formation of biofilms on the biomaterial surface and colonization of the surrounding tissue. Due to the combined presence of biomaterial and bacteria the local immune response is compromised, leading to inability of host immune cells to kill phagocytosed bacteria. Direct treatment of these infections by antibiotics is difficult due to the localization of the bacteria, and their low metabolic state. Moreover, antibiotic resistance is an increasing problem.
In order to prevent biomaterial associated infection, novel antimicrobials are required which (i) have microbicidal activity, (ii) prevent biofilm formation, (iii) prevent the immune-dysregulating inflammatory activity of bacterial compounds, (iv) do not have a risk for resistance development, and (v) are active human plasma. In addition, the antimicrobials need to be released from the surface of biomaterials at sufficient concentrations and for a sufficientperiod to prevent infection.
In the EU project BALI we have therefore developed novel Synthetic Antimicrobial Antibiofilm Peptides (SAAPs) based on the primary structures of the antimicrobial proteins LL-37 and thrombocidin-1. The peptides kill a wide spectrum of Gram-positive and -negative (antibiotic resistant) bacteria at concentrations ranging from 0.8 - 8 µM in PBS. In presence of 50% human plasma the bactericidal concentrations are 2 – 32-fold higher, depending on the peptide and tested strain. The SAAPs prevented biofilm formation of *Staphylococcus aureus* at concentrations of 3.2 – 12.8 µM. They also had potent anti-inflammatory activity: they inhibited production of IL-12 and IL-8 by cells in whole blood upon stimulation with lipopolysaccharide (LPS) and UV-killed *S. aureus.*
The different SAAPs were eluted from innovative coatings designed using pharmaceutically approved polymers and lipids (PolyPid), tailored to accommodate an initial high rate short term release in the first days, and subsequent zero-order kinetic release over approximately 30 days. The coatings applied on titanium implants reduced the numbers of *S. aureus* colonizing the implant after 1 day in the mouse subcutaneous biomaterial–associated infection model. Long term experiments in this model and in a rabbit humerus intramedullary nail infection model are currently undertaken. Thus, the promising characteristics and activity of the SAAPs and their controlled release coating, both developed in BALI, indicate a strong potential to “beat biofilms”.

Notes:

Antimicrobial activity of synthetic peptides and lipopeptides derived from lactoferricin against *Pseudomonas aeruginosa* biofilms

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Infections by *Pseudomonas aeruginosa* constitute a serious health threat because this pathogen – particularly when it forms biofilms - can acquire resistance to the majority of conventional antibiotics. This study evaluated the antimicrobial activity of synthetic peptides based on LF11, an 11-mer peptide derived from human lactoferricin against *P. aeruginosa* planktonic and biofilm-forming cells. We included in this analysis selected N-acylated derivatives of the peptides to analyze the effect of acylation in antimicrobial activity. To assess the efficacy of compounds against planktonic bacteria, microdilution assays to determine the minimal inhibitory concentration (MIC) and time-kill studies were conducted. The anti-biofilm activity of the agents was assessed on biofilms grown under static (on microplates) and dynamic (in a CDC-reactor) flow regimes.
We demonstrated that peptides differed from lipopeptides in their killing mechanisms on planktonic cells: whereas some peptides rendered the lowest MICs, lipopeptides killed much more rapidly than their parental compounds. In general, acylation resulted in compounds with lower anti-biofilm activity. In contrast, two peptides, LF11-215 and LF11-227, displayed the most potent anti-biofilm activity causing a 10,000 fold reduction in cell viability after 1h of treatment while the latter agent removed more that 50% of the biofilm mass. Peptide LF11-215 and two lipopeptides (the least hydrophobic), DI-MOLF11-233 and DI-MO-LF11-215, penetrated deep into the biofilm structure and homogenously killed biofilm-forming bacteria. These results indicate that lactoferricin derived peptides are promising anti-biofilm agents. Further structure-activity relationship analyses are necessary to optimize the anti-biofilm activity of lipopeptides.

Notes:

Influence of lipopeptides with two fatty acid chains on bacterial biofilm

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Numerous microorganisms show the ability to form biofilms on human tissues and biomaterials. These highly specialized structures demonstrate a high resistance level to the vast majority of antibiotics leading to severe therapeutic complications.
Among the most extensively investigated potential antibiofilm agents are antimicrobial peptides (AMPs). The activity of AMPs against drug-resistant microorganisms, both in planctonic, as well as in the biofilm forms is well known.
The purpose of this study was to synthesize a group of short cationic lipopeptide similar in structure to AMPs, but instead of well-defined amino acid hydrophobic region fatty acid residues were introduced. The lipopeptides: $(C_8)_2$-KKKK-NH_2, $(C_{10})_2$-KKKK-NH_2, $(C_{12})_2$-KKKK-NH_2 and $(C_{14})_2$-KKKK-NH_2) were synthesized by the solid-phase procedure using 9-fluorenylmetoxycarbonyl (Fmoc) metodology and purified by solid phase extraction (SPE) with octadecyl RP phase.
Minimum Inhibitory Concentration (MIC) and Minimum Biofilm Eradication Concentration (MBEC) were determined on reference strains of Gram-positive (*Staphylococcus aureus*, *S. epidermidis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria. The influence of lipopeptides on biofilm formation was also tested. Crystal violet was used for biofilm quantification.
The data demonstrate that all lipopeptides inhibit the growth of test bacteria, while most active are the analogues $(C_{10})_2$-KKKK-NH_2 and $(C_{12})_2$-KKKK-NH_2. Tested compounds exhibited strong antibiofilm activity as their MBECs were only few times higher in comparison to MICs. Moreover the application of lipopeptides at concentrations below their MICs was sufficient to significantly reduce or inhibit the formation of biofilm.

Notes:

Synergistic activity of peptides and common antifungal drugs on biofilms

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Biofilm formation of fungal pathogens like *Candida* spp. is a key process in many fungal infections. Implanted medical devices like intravascular catheters, orthopaedic joints or heart valves are a preferred niche for *C. albicans* cell adherence and biofilm formation. Unfortunately, most antifungal drugs show only limited antibiofilm activity, with echinocandins (e.g. caspofungin) and liposomal formulations of amphotericin B being the most effective. Hence, past years, we have screened for small molecules and natural peptides, so called potentiators or enhancers that can increase the antibiofilm activity of standard antimycotics. Examples of such peptide potentiators are the bacterial cationic cyclodecapeptide tyrocidines, the plant decapeptide OSIP108, and a 14-AA CRAMP peptide fragment, termed AS10. We have shown that all these peptides can increase the *in vitro* antibiofilm activity of amphotericin B and/or caspofungin against *C. albicans* biofilm formation and/or biofilm eradication, without displaying cytotoxicity against human cell lines. Furthermore, we demonstrated that tyrocidine A and OSIP108 both enhance the antibiofilm activity of caspofungin *in vivo* in a *Caenorhabditis elegans*-*C. albicans* biofilm infection model. These data point to the potential use of peptides in combination therapy with conventional antifungal agents to combat fungal biofilm-related infections.

Notes:

Novel class of peptides derived from a protein sequence of *Candida albicans* Als3 reduced attachment to plastic, mature biofilm development, adhesion and invasion to human epithelial cell lines.

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Candida albicans biofilm-associated infections represent a medical problem, due to the increased resistance of the cell population within biofilms against antifungals. Therefore antifungal research is focused mainly on development of novel technologies leading to the generation of novel therapeutics. Herewith, we used TANGO algorithm (Fernandez-Escamilla *et al*., 2004) to predict sequences of Als3 with a high propensity to cause *ß*-aggregation. *C. albicans* Als3, a GPI-anchored protein covalently bound to the cell wall has an important role in biofilm formation *in vitro*, as well as in adhesion and invasion to human tissues. Corresponding short (maximum 20 amino acids) peptides were designed, synthesized and added to *C. albicans* thereby reducing adhesion and subsequently diminishing *in vitro* and *in vivo* biofilm formation. Additionally, peptide-treated *Candida* cells displayed reduced adhesion and invasion into human cells. The underlying mechanism of a peptide is unknown but it might potentially cause aggregation of the target Als3, as FACS analysis and atomic force microscopy clearly shows less "functional"' Als3 present on the hyphal cells. Results obtained in this study are very promising for development of peptide-based therapeutics.

Fernandez-Escamilla A. M., Rousseau F., Schymkowitz J. and Serrano L. 2004. Prediction of sequence-dependent and mutational effects on the aggregation of peptides and proteins. Nat Biotechnol 22:1302-1306.

Notes:

Characterizing the curli fimbriae on living bacterial surfaces using scanning probe microscopy and single molecular force spectroscopy

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The microbial functional amyloids called curli are the major feature of complex extracellular materials (ECM) of *E. coli strains* and implicated in many physiological and pathological processes of *E. coli*, including epithelial attachment and tissue invasion. Recent research indicates that amyloids of human and animal pathogens might facilitate colonization of host tissue by binding the ECM component fibronectin and subsequently activating the fibrionlytic and contact systems of haemostasis [1]. In a recent study we used scanning probe microscopy to determine the effects of curli on the topology and on the mechanical properties of live *E. coli* cells [2]. Here, so as to identify and characterize the curli binding site on fibronectin, various fibronectin constructs varying in size were coupled onto silanized silicon nitride AFM tips employing specially tailored chemistry protocols. Single-molecular force spectroscopy revealed specific interaction forces between CsgA on the living bacterial surface and fibronectin. When bacteria produced CsgA curli proteins on their surface, they bound not only to the full length of fibronectin but also to fibronectin domain III and RGD peptide, whereas CsgA knock-out mutant showed much lower binding activities. Alternative configurations of force measurement modes, such as bacteria coupled to the tipless AFM cantilever and fibronectin adsorbed to the substrate, were used for comparison. As the contact area between bacteria and fibronectin increased in the latter configuration, CsgA over expressed mutant showed forces of a few nN ranges arising from multiple molecular interactions. Our results elucidate the complex multiple adhesive properties of curli-mediated bacterial interaction with fibronectin.

References

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Notes:

Insect-derived proline-rich antimicrobial peptides: Novel protein targets and transport mechanisms

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The health threat of multidrug resistant bacterial pathogens demands antibiotics killing bacteria by novel mechanisms. Antimicrobial peptides (AMPs) represent such a promising class of new compounds, especially as they have already proven their efficacy as part of innate immunity. Our research focuses on proline-rich AMPs, i.e. apidaecin (honeybees) and oncocins (milkweed bugs), aiming at optimized compounds for systemic treatments of Gram-negative infections including sepsis. Lead compounds Api88 and Api137 (apidaecin analogs) as well as oncocins Onc72 and Onc112 are highly active against *Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*. Importantly, they are well tolerated in mice and highly efficient in *Escherichia coli* infection models with Api137 doses of 0.64 mg/kg rescuing all animals in a lethal systemic septicaemia infection model. Their *in vivo* efficacy was further confirmed in a murine infection model established for multi-resistant *K. pneumoniae*.

As the mode of action is still unclear, we have used affinity chromatography and photo-crosslinking to identify bacterial protein binding partners of Api88 and Onc72. The first approach revealed several proteins, though the enrichment was not very reproducible and thus the identification of potential targets questionable. The second strategy provided many potential binding partners including proteins of the 50S- and 30S-ribosome. Further studies revealed that apidaecin- and oncocin-derived analogs bound strongly to the ribosome ($K_d \leq 1$ µmol/L) and that oncocins inhibit protein translation with IC_{50}-values in the nmolar-range. Positional mapping identified several residues in the N-terminal region of oncocin and the C-terminal region of apidaecin that appear to be responsible for the strong interactions.

Further studies revealed also potential resistance mechanisms in *E. coli*. Importantly, the resistance mechanisms of *E. coli* should significantly reduce the virulence of these strains and thus not represent a major health threat.

Notes:

Pharmacokinetics and Tissue Distribution of the proline-rich antimicrobial Peptide Onc72

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Since the 1930s bacterial infections have been treated efficiently with different classes of antibiotics subsequently introduced into the market. However, single and multi-resistant strains were favored by partially uncontrolled and unnecessary treatments provoking serve health concerns. Efficient antimicrobial compounds lost more and more their potency leading to an increased demand for new chemical lead compounds [1]. The family of proline-rich antimicrobial peptides (PrAMP) is mostly active against gram-negative bacteria. Due to their non-lytic mechanism targeting intracellular components, they represent a very promising group of novel antibiotics [2]. Furthermore, NMRI mice treated with our lead compound oncocin Onc72 (VDKPPYLPRPRPPROIYNO-NH_2) showed no signs of acute toxicty for a daily dose of 160 mg/kg. In a model of intraperitoneal sepsis with *E. coli* ATCC 25922 a 20 times lower daily dose provided a survival rate of 50 % (ED_{50} = 7.5 mg/kg) [3].
In order to establish a sensitive, fast, and reproducible sandwich ELISA for pharmacokinetics and tissue distribution studies (kidney, liver, brain) of Onc72, we immunized BALB/c mice to generate specific monoclonal antibodies (mAbs). Since native Onc72 was not immunogenic in mice after administration over 7 weeks, it was coupled to keyhole limpet hemocyanin (KLH) for successful immunization. Finally Onc72 was also detected in urine.

Key words: Antimicrobial Peptide, Sandwich ELISA, Pharmacokinetics

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Notes:

Chitosan Nanoparticles for the Linear Release of Cationic Antimicrobial Peptides

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Despite an impressive antimicrobial activity, the therapeutic potential of the Antimicrobial peptides (AMPs) is mainly hampered by their fast degradation in biological fluids. Encapsulation of AMPs into polymeric nanoparticle (NPs) provides better stability of the peptides and allow its delivery to eukaryotic and prokaryotic microorganisms, growing as planktonic cells as well as biofilms. In particular, the development of drug delivery systems (DDS) with linear drug releasing profiles and tunable release rates is generally considered of best importance for the optimization of therapeutic regimens.
The present study is focused on the development of nanosized DDS for the linear release of amphipathic cationic AMPs, starting from preliminary investigations of the loading of Lysozyme into chitosan based NPs (CS-NPs) and ending with the assessment of the antimicrobial properties of CS-NPs loaded with Temporin 1b (T-1b). Beyond the intrinsic antibacterial activity of CS-NPs and T-1b, the nanocarrier evidenced a sustained action against *Staphylococcus epidermidis* for at least 4 days. At this time point, the results showed almost 4-log reduction of the number of viable *S. epidermidis* compared to plain CS-NPs and 6-log reduction compared to plain T-1b. Furthermore, the nanocarrier showed full *in vitro* cytocompatibility toward murine fibroblasts.
In addition to T-1b, the developed nanocarrier is eligible for the administration of several AMPs of therapeutic interest comprising temporin and japonicin-1 families, meucin peptides, mastoparan peptides, and other synthetic AMPs with analogous physical characteristics. Their evaluation is presently ongoing.

Notes:

In ovo administration of the chicken cathelicidin peptide analog DCATH-2 protects against bacterial infection in young broilers

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Mammalian host defense peptides (HDPs) exhibit a wide array of immunomodulatory functions on immune cells, including apoptosis inhibition, recruitment, wound healing, enhanced antigen uptake and presentation and may so boost innate immunity. This study was aimed to determine *in ovo* efficacy of chicken HDP analog DCATH-2 to reduce experimentally induced salmonellosis in young broiler chickens. First, we studied the uptake of ^{89}Zr-radiolabeled CATH2 peptide via the amnion of 18-day old embryos by positron emission and computed tomography (MicroPET/CT) and by *ex vivo* biodistribution analysis. These showed a significant uptake of peptide in stomach, lungs and intestine within 24 hours. Next, an efficacy study was performed by injecting DCATH-2 (1mg/kg bodyweight) into the amnion of 18 day-old Ross 308 embryos that were challenged subcutaneously at 4 days post hatch with 5×10^6 CFU of avian pathogenic *Salmonella enteritidis* PT13a. DCATH-2 treatment increased peripheral blood leukocyte and heterophil counts at 48h p.i. and greatly reduced mortality (50%), the number of birds with lesions (69%) and lesion severity (67%) at 7 days p.i. Similar results were obtained in a model with an intratracheal *E. coli* challenge of broilers 7 days post-hatch. These findings show that *in ovo* administration of HDP-based anti-infectives is effective in prevention of post-hatch bacterial infection of broilers and has potential for use in veterinary medicine.

Notes:

Optimizing the therapeutic potential of short antimicrobial peptides

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Despite decades of intensive research, antimicrobial peptides (AMPs) have not yet revealed all their secrets; in fact, increasingly they are appearing to be more complex than previously imagined. In recent years, it has become clear that they are not only able to kill Gram-positive and Gram-negative bacteria, fungi, parasites and enveloped viruses, but can also alter immune response in mammals. They have been used successfully in animal models, for the prevention of septic shock, they have been shown to be chemotactic, promoting wound healing and angiogenesis, and they have been found to selectively modulate chemokine and cytokine production; however, the mode of action of these AMPs, especially short peptides with a length between 9-13 amino acids, are not yet understood. Little is known about the sequence requirements of short cationic AMPs. With help of our novel technique using an artificially created luminescence producing Gram negative bacterium and peptide synthesis on cellulose (SPOT technology), we investigated the sequence requirements of such peptides. Several thousands of peptides were tested for their ability to kill *Pseudomonas aeruginosa*. The data is being analyzed using a specifically adapted program. In addition to the antimicrobial activity the hemolytic activity of 3600 peptides were determined. We are now able to predict active peptides that are harmless against human erythrocytes and therefore optimize the therapeutic index. The results of these measurements and analyses will be discussed in detail.

Notes:

Posters:

Abstracts

Poster 1:

*pal*G1: A novel antimicrobial from cow rumen

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The development of antibiotic molecules for use in the treatment of infections and for the upstream control of contamination has been declining over recent decades. Urgent action is needed to address this problem which is further compounded by the evolution of antibiotic resistance. Vancomycin-resistant *Enterococci* (VRE) have emerged as a major cause of nosocomial infections, and within this group *Enterococcus faecalis* causes the majority of human enterococcal infections.
In this study, a novel antimicrobial peptide ***pal*G1** discovered by functional and sequence based screening of a fosmid-based cow rumen metagenomic library was successfully expressed and tested for activity against *Enterococcus faecalis* JH2-2. Growth of *E. faecalis* in Luria Bertani broth in the presence of ***pal*G1** was monitored over a 24 hour period. Bacterial growth was inhibited by ***pal*G1** (100 μg/ml) compared to medium with bacteria alone. Although, still in the early stages of characterisation, current results clearly demonstrate that ***pal*G1** has potential for use in the treatment of enterococcal infections, and the rumen is a potential a source of novel antimicrobial peptides that could provide templates for the design of novel therapeutics in the future.

Notes:

Poster 2:

An antimicrobial peptide library screen against *Candida albicans*

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Fungal infections are exceptionally hard to eradicate and contribute to the high number of deaths among immunocompromised patients. The most common fungal infections are caused by the yeast *Candida albicans*. These infections are difficult to treat and overall the choice of currently available treatment options is very restricted. Additionally, resistance is spreading amongst fungal strains and compromises the small arsenal of antifungal drugs In the light of the developing drug resistance among fungal strains novel approaches are urgently needed. Antimicrobial peptides are gaining popularity as a potentially new option that can offer a solution to the problem In search of more potent, non-toxic compounds we tested a repertoire of 600 9mer antimicrobial peptides against *Candida albicans*.

Notes:

Poster 3:

Mode-of-action of antifungal peptides and peptidomimetics

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The significance of fungal infections has been grossly underestimated. Only a few drugs are clinically available to treat life-threatening fungal infections, and resistance against these drugs is rising. Natural and synthetic antifungal peptides (AFPs) are being actively explored as novel pharmaceuticals. PAF26 is a *de novo*-designed hexapeptide possessing two well-defined motifs: a N-terminal cationic region and a C-terminal hydrophobic region. We have analysed the role of each motif in the antifungal mode-of-action of PAF26, and our results show that PAF26 has a dynamic antifungal mode-of-action that involves three stages: electrostatic interaction with cell membrane, internalisation, and cell killing[1,2]. However, the mechanism by which it causes fungal cell death is unclear. In order to obtain further insights into its mode-of-action, a library of PAF26 labelled with 14 different fluorophores has been synthesised and tested. This library covers both broad chemical and spectral diversity, and is currently analysed by live cell imaging in the fungal model *Neurospora crassa* and the human fungal pathogen *Aspergillus fumigatus*. This study will provide novel mechanistic insights in the mode-of-action of PAF26 and will facilitate the design of new peptides, peptoids and other peptidomimetics with improved antifungal activity and stability.

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Notes:

Poster 4:

Transport across the bacterial membrane and effect of length on the internalisation of the proline-rich peptide Bac7

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Bac7 is a proline-rich antimicrobial peptide, active mainly against Gram-negative bacteria, whose antimicrobial effect is related to its capacity of being internalised into the bacterial cytoplasm without membrane damage. The translocation of Bac7 across the *E. coli* inner membrane is mediated by the SbmA protein, which is a dimeric proton-driven transporter found in distantly related species of Gram-negative bacteria.
In the present work we showed that Bac7 directly binds to the SbmA protein *in vitro* and is efficiently transported by the protein *in vivo* in a whole cell transport assay with a k_m value of 6.95 ± 0.89 μM peptide and a V_{max} of 53.91 ± 3.17 nmol/min/mg SbmA. Moreover we showed that progressively shortened fragments of Bac7 exhibit a parallel decrease in their internalisation levels and we determined the minimal sequence length required for entry as a 16-residue, N-terminal fragment.
Taken together these results shed light on the transport parameters of Bac7 and on the close relationship existing between sequence length and internalisation in the bacterial cell, representing a first step towards the optimization of this peptide as a carrier for the transport of other cargo molecules.

Notes:

Poster 5:

Oncocin and apidaecin derivatives bind to the *Escherichia coli* 70S ribosome and inhibit the protein translation *in vitro*

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Keywords: apidaecin, oncocin, PrAMPs, protein translation, ribosomes

Proline-rich antimicrobial peptides (PrAMPs) have been investigated and optimized by several research groups and companies as promising lead compounds to treat systemic infections caused by Gram-negative bacteria. PrAMPs, such as oncocins and apidaecins, kill Gram-negative bacteria (e.g. *Escherichia coli* and *Klebsiella pneumonia*) by a non-lytic mechanism. They enter the periplasm by interaction with the negatively charged bacterial outer membrane and are transported by the SbmA transporter into the cytosol.[1] Their activity is mediated by binding to intracellular targets, such as the well studied chaperone DnaK.[2] However, previous and current studies of our group challenged DnaK as the major target and indicated that PrAMPs bind to the 70S ribosome and might thereby interfere with protein translation.[3,4,5]
We were able to show, that oncocin and apidaecin derivatives bind with nanomolar dissociation constants (K_d) to the 70S ribosome. In oncocins, the N-terminal residues Lys3, Tyr6, Leu7, and Arg11 are the major interaction sites with the 70s ribosome, whereas apidaecins interact strongly with the C-terminal arginine residue Arg17. Oncocins inhibited the protein biosynthesis very efficiently *in vitro* with half maximal inhibitory concentrations (IC_{50}) of 150 to 240 nmol/L. The apidaecin derivatives showed a steady decrease of the *in vitro* protein expression down to approximately 40%.

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[5] Krizsan *et al.* (2014), Angew. Chem. Int. Ed., in press (DOI: 10.1002/anie.201407145)

Notes:

Poster 6:

Pharmacokinetics of proline-rich antimicrobial peptides

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Antimicrobial peptides (AMPs) represent a promising class of new antibiotic agents to treat the growing number of multidrug resistant bacteria. Peptides Api88 and Api137 as well as Onc72 and Onc112, which were rationally optimized from apidaecin 1b[1] (identified in honey bees) and oncocin[2] (milkweed bugs), respectively, are highly active against Gram-negative pathogens[3,4] both *in vitro* and *in vivo* despite huge differences in their serum stabilities. Thus, we studied their pharmacokinetics in mice after intravenous (*i.v.*) and intraperitoneal (*i.p.*) injection in blood, urine, and different organs. The full length peptides and their major degradation products were enriched by solid phase extraction (SPE) and quantified by RP-HPLC-ESI-MS/MS using isotope labeled standard peptides and multi-reaction-monitoring (MRM).
After *i.p.* administration of single peptide doses of 5 and 20 mg/kg body weight (BW) the highest oncocin plasma levels (C_{max}) were observed with 17 µg/mL at 10 min post injection (*p.i.*). At this time point the concentrations of the apidaecin peptides were significantly lower (C_{max} ~ 4 µg/mL), with considerably higher concentrations of truncated sequences (metabolites) compared to the oncocins. Surprisingly, the *in vivo* half-life of oncocins and apidaecins were similar (around 10 to 20 min) for the high dose groups. Peptide concentrations in kidney and liver homogenates were 1.5 µg/g and 0.2 µg/g, respectively. According to these results a renal clearance seemed to be preferred and was further confirmed by urine containing these peptides at concentrations above 0.2 µg/mL for mice treated with 20 mg/kg BW.

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Notes:

Poster 7:

Design of PEGylated prodrugs providing ideal release-kinetics of peptides

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Peptide researchers have isolated, optimized, and rationally designed many compounds with superior *in vitro* activities. Many of these peptides, however, showed unfavorable pharmacokinetics owing to fast renal clearance or proteolytic degradation. Some promising peptides were rescued by permanent coupling of synthetic polymers, such as polyethylene glycol (PEG). Besides reduced renal clearance rates PEGylation often diminishes also toxic and immunogenic effects. Many compounds, however, are also inactivated by irreversible PEGylation, especially when targeting intracellular structures. Therefore, a new type of prodrug technology was developed in order to release the active compound *in vivo*. The drug is attached to PEG via a short peptide linker, bearing a cleavage site for endogenous proteases. Variation of peptide linker length and sequence enables individual tuning of the release kinetics and thus its concentration in blood to provide an ideal pharmacokinetics for each drug. This strategy has been applied to antimicrobial peptides apidaecin and oncocin that were significantly stabilized without loss of activity after appropriate release. In this study, we investigated the fine-tuning of the release kinetics and developed a set of linker sequences that provide various protease release rates spanning one order of magnitude. Furthermore, we investigated the influence of the peptide-PEG ligation strategy on prodrug stability.

Keywords: Antimicrobial peptides, controlled release, prodrug, reversible PEGylation

Notes:

Poster 8:

Can minimal inhibitory concentrations predict the *in vivo* efficacy of proline-rich antimicrobial peptides?

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Proline-rich antimicrobial peptides (PrAMPs) are promising lead structures for the development of new antibiotics to treat multidrug resistant infections. They are especially active against Gram-negative bacteria, such as *Escherichia coli*, *Klebsiella pneumoniae*, and the non-fermenters *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Unfortunately, antimicrobial activities depend strongly on the medium concentration in which the minimal inhibitory concentrations (MIC) are determined according to CLSI standard methods. The MIC values of optimized oncocin (Onc72, Onc110, and Onc112) and apidaecin derivatives (Api88, Api134, and Api137) against *E. coli* and *K. pneumoniae* in full media are typically 16 to 32 µg/mL. Such concentrations are difficult to maintain in pharmacokinetic studies raising concerns about the efficacy of these peptides *in vivo*. In diluted media, however, the antimicrobial activities increase to 2 to 16 µg/mL. Thus the peptides were evaluated in two murine systemic septicemia models initiated by intraperitoneal infection with *E. coli* ATCC 25922 and a Carbapenemase producing *K. pneumoniae* strain.[1,2] Effective doses for 50% survival (ED_{50}) were determined between 0.6 and 5 mg/kg body weight (BW) applying intraperitoneal treatment.[3] Additionally, oncocin Onc72 and apidaecin Api88 protected mice in a thigh abscess mouse model with *K. pneumoniae* using subcutaneous injections.

[1] Knappe D. et al., *J. Antimic. Chemotherapy*, 2012
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Notes:

Poster 9:

Toward optimization of antimicrobial peptides for the treatment of multidrug resistant infections in cystic fibrosis.

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Patients with cystic fibrosis (CF) often require pharmacological treatment against problematic chronic lung infections due to antibiotic-resistant strains. A strategy to overcome this concern may be the use of Antimicrobial Peptides (AMPs). Although several AMPs are active *in vitro* against resistant strains and can eradicate or counteract the formation of biofilms, they often show acute toxicity when administrated *in vivo*. Our aim was to develop some modified forms of natural cathelicidins, in order to retain a good bactericidal activity while reducing the effects of toxicity toward the host. BMAP-27 (1-18), BMAP28 (1-18) and mBMAP28 have been synthesized and their activity against 45 CF strains (15 each of *P. aeruginosa*, *S. aureus*, and *S. maltophilia*) was assessed. The acute toxicity at pulmonary level of AMPs was also evaluated in a mouse model following intratracheal instillation. All AMPs have shown to maintain a good antibacterial activity *in vitro*, when compared to the natural molecules. Toxicity assays *in vivo* showed that BMAP-27 (1-18) was the less toxic among the tested peptides. Brought together, results from antimicrobial activity and lung toxicity showed BMAP-27 (1-18) as the best peptide to be tested for *in vivo* activity in a murine model of acute lung infection by *P. aeruginosa*. In spite of promising *in vitro* antibacterial activity, BMAP-27 (1-18) did not show the desired curative effect *in vivo*. These results indicate that further studies are necessary on the route of administration, and to reduce the toxicity in order to optimize BMAP-27 (1-18) for the pulmonary environment.

Notes:

Poster 10:

Discovering features of low toxicity peptides that predict killing of MRSA

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We currently face a threat from multi drug resistant pathogens, if not addressed then in proceeding years infections that were once easy to treat will be reclassified as life threating illnesses. Furthermore the antibiotic reliance inherent in numerous medical procedures will mean former routine procedures will become far more dangerous or perhaps too risky altogether. In order to maintain the upper hand in the eternal fight between pathogen and host, we need to find antimicrobials that are less prone to resistance development and which can act as the lead compounds for a variety of different drugs. Antimicrobial peptides are versatile lead compounds that can be modified to an incredibly high degree; furthermore numerous studies have found that when compared to many conventional drugs antimicrobial peptides are far less likely to induce antimicrobial resistance. The problem is that with so many possible combinations of amino acids and other chemical moieties how can we find the optimal structures to kill pathogens and simultaneously be non-toxic to the host. Here we use a method of high throughput synthesis and screening to measure the antimicrobial activity towards MRSA of 600 peptides that have been predicted in silico to be non-toxic to red blood cells. Furthermore we tested the validity of the prediction by measuring the haemolytic activity of all 600 peptides. The combination of antimicrobial and toxicity data will be used to predict a new panel of peptides that are both highly active towards MRSA and yet maintain low toxicity.

Notes:

Poster 11:

Polymyxin – long forgotten weapon against endotoxemia

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In recent years, endotoxins (lipopolysaccharides, LPS) have become more and more one of the main targets in diseases that are treated with extracorporeal blood purification. LPS is a major constituent of the outer cell wall of gram-negative bacteria and strongly triggers inflammatory responses in humans at concentrations as low as 1 ng/kg body weight per hour (1). Although there is an urgent need for endotoxin elimination not only for the treatment of sepsis, but also for liver failure, endotoxin neutralization studies using endotoxin adsorbents have been disappointing. Only Toraymyxin®, a clinical approved Polymyxin B (PMB) based adsorbents, shows promising results for endotoxin elimination (2), but our recent in vitro experiments suggest that part of the effect on LPS inactivation of this adsorbent might be achieved by the release of PMB from the carrier into the surrounding medium, which neutralizes the biological activity of LPS (3). Here we show that endotoxin inactivation could be applied by intravenous infusion of PMB in patients with gramnegative sepsis or endotoxemia. According to in-vitro measurements of LAL activity and cytokine release from blood cells, the optimal PMB plasma concentration for LPS inactivation is in the range of 100 and 200 ng/ml. Endotoxin inactivation by low dose intravenous PMB infusion or infusion into the extracorporeal circuit during blood purification treatments could be applied to overcome the urgent need for endotoxin elimination not only in treatment of sepsis, but also in liver failure.

Notes:

Poster 12:

Stimulation of human monocytes with bacterial and viral components increases the expression of hepcidin

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Hepcidin belongs to the antimicrobial peptide (AMP) family and is the key regulator of iron metabolism. It modulates iron homeostasis by binding to, and degrading the iron exporter molecule ferroportin thus inhibiting cellular iron efflux. Many antimicrobial peptides have a dual function; some are able to act directly as an antimicrobial agent as well as having an immunoregulatory role in the host.
Toll-like receptors (TLRs) bind to components of microbes, activate cellular signal transduction pathways and stimulate innate immune responses. The effect of viral and bacterial TLR co-stimulation of THP-1 derived monocytes showed that 24 hours after exposure to TLR 9 and TLR3 agonists in combination, hepcidin expression was significantly increased (10 fold) when compared to the untreated control. This combination of TLR ligands mimics simultaneous bacterial and viral infections thus suggesting a potential key role for hepcidin in combined infections.
Additionally, by using a chequerboard assay, we have shown that hepcidin has an antagonistic effect in combination with the antibiotics rifampicin and tetracycline against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus pyogenes*, evidenced by a fractional inhibitory concentration index (FICI) >4. This finding has important implications for future treatment regimens especially in an era of increasing antimicrobial resistance.

Notes:

Poster 13:

Synergistic activity of antimicrobial peptides secreted from frog skin

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The increase of pathogens being resistant to antibiotics represents a global health problem and thus, there is an urgent need for the development of antibiotics with novel mechanisms of action. Naturally occurring antimicrobial peptides, which interfere physically with cell membranes, are valuable template structures. In various organisms they are often synthesized in conjunction with a number of other peptides suggesting that these peptides may act synergistically. For example, various biophysical studies on the frog skin antimicrobial peptides magainin 2 and PGLa indicated a synergistic activity by membrane pore formation (1,2). However, recent studies showed that magainin 2 remains surface-bound independent on the lipid matrix, while PGLa was able to adopt a tilted or transmembrane orientation depending on the nature of the lipid (3,4). Therefore, we have addressed the question, if an equimolar ratio of peptides is a necessity for their synergistic activity.
Peptide mixtures of different molar ratios showed higher permeabilization activity on fluorescent dye loaded POPG/POPE-liposomes mimicking bacterial membranes than the individual peptides at the same concentration, whereby an equimolar mixture of magainin 2 and PGLa as well as the hybrid peptide linked at the C-termini were most effective. Selected samples were further analyzed in respect to thermodynamic and structural parameters to gain information on the mechanism of membrane perturbation. A first set of experiments revealed peptide induced segregation of a POPE-enriched phase, again being more prominent in the presence of an equimolar peptide mixture and the hybrid peptide causing the formation of a second liposomal population. Such insight will not be only fundamental to unravel the case of PGLa/magainin 2 synergism but also for other peptide combinations in general.

(1) Nishida M. et al. Biochemistry 46 (2007)14284-14290.
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(3) Salnikov E.S. & Bechinger B . Biophys. J 100 (2011) 1473-1480.
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Notes:

Poster 14:

Human Lactoferricin derivatives as new weapons in cancer therapy

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Despite favorable advancements, cancer is still a leading cause of death. Weak cancer toxicity or side effects are caused by inadequate specificity for cancer cells. In our study derivatives of a short cationic peptide derived from the human host defense peptide lactoferricin were further optimized in their activity and selectivity towards cancer cells. The target of the peptides is the negatively charged membrane lipid phosphatidylserine (PS), specifically exposed by cancer cells during malignant transformation (1).
Peptide-membrane interactions were studied using various biophysical methods like differential scanning calorimetry, fluorescence and circular dichroism spectroscopy and fluorescence microscopy. PS and PC liposomes, mimicking the cancer and non-cancer cell membrane, respectively, were used to study the influence of peptides on the respective lipids. Further, we demonstrated that hLFcin derivatives exhibit anticancer activity *in vitro* against malignant melanoma, metastasis thereof, and glioblastoma, correlating with selective activity against the cancer model PS. Additionally, peptide effects on non-tumorigenic melanocytes and human dermal fibroblast cells were studied.
The peptides differed in several aspects: length, net charge, hydrophobicity, and consequently secondary structure. Short peptides were only minor active. Elongation of certain peptide sequences led to highly active and selective peptides with more than 20-fold specificity for cancer cells (2). Regarding cancer cell selectivity, adoption of a certain structural conformation in presence of the target membrane and the killing mechanism seem to play an important role.

Acknowledgment: Austrian Science Foundation FWF (grant no. P20760, P24608)

(1) Riedl et al. BBA 1808: 2638-2645, 2011
(2) Riedl et al. BioMetals 27(5): 981-997, 2014

Notes:

Poster 15:

Biological and physicochemical properties of short lipopeptides

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Cationic lipopeptides are nowadays widely studied due to their high antimicrobial potential. Taking into account the amphiphilic structure it is understandable that lipopeptides often demonstrate self-assembling abilities.
The purpose of this study was to synthesize a group of short cationic lipopeptides with surfactant-like construction containing two hydrophobic fatty acids chains. Compounds were subjected to microbiological tests: MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concentration). The haemolytic activity of the lipopeptides was also determined. The self-assembling tendency was estimated by the critical micelle concentration studies.
The data demonstrate that, among the five lipopeptides, the most active are the analogues $(C_{10})_2$-KKKK-NH_2 and $(C_{12})_2$-KKKK-NH_2. They already inhibit the growth of *B. subtillis* at a concentration of 2 μg/mL and of *S. epidermidis* at 2 and 4 μg/mL, respectively. The cationic lysine-derived surfactants are characterized by different haemolytic properties. However all tested lipopeptides at concentrations of 2 μg/mL caused haemolysis less than 3%. Above this concentration haemolytic activity of four compounds consecutively increases but in non-linear manner. The micellization process for amino acid based surfactants containing two hydrophobic chains strongly depends on the length of the hydrophobic chains attached to the α and ε amino groups. The CMC decreases with an increase in the alkyl chain. The selected lipopeptide surfactants studied in this work exhibited excellent antimicrobial properties. It was found that the studied compounds displayed a weaker activity against Gram-negative strains and fungi than against Gram-positive bacteria.

Notes:

Poster 16:

Temperature dependence of L_o/L_d domain thickness and elasticity by global SAXS data analysis

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The spatial organization of lipids in biological membranes plays an important role in diverse cellular processes. Of particular interest are membrane rafts which are considered to enable cellular signaling and transport. Such membrane rafts are currently mimicked by liquid ordered (L_o) domains, observed in several lipid-only mixtures. The physical properties of such L_o domains and the coexisting liquid disordered (L_d) phase are presently not well known. We developed a global small-angle x-ray scattering data analysis for multilamellar vesicles that allows to determine membrane structural parameters and bending fluctuations of coexisting lipid domains. Different scattering length density profiles were used for modeling the lipid bilayers with increasing details and complexity. The technique was applied to different ternary and quarternary phospholipid mixtures of DOPC/DPPC/Chol, DOPC/DSPC/Chol and DOPC/DSPC/POPC/Chol. We determined membrane thickness as well as bending elasticity for L_o and L_d phases as a function of lipid composition and temperature. In particular, we discuss changes in these parameters upon the transition to a homogeneous phase.

This work is supported by the Austrian Science Funds FWF, Project No. P24459.

Notes:

Poster 17:

Protein partitioning in liquid-ordered (L_o) / liquid-disordered (L_d) domains depends on lipid composition and protein shape

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The lack of transmembrane proteins partitioned in the current lipid-only models for membrane rafts, i.e. Lo phases, calls for close scrutiny of raft mimetics. Using small angle X-ray scattering (SAXS) and molecular dynamic simulations (MD), we determined structural and elastic parameters (spontaneous curvature, bending rigidity, Gaussian curvature modulus) for coexisting L_o/L_d domains in ternary mixtures of dioleoylphosphatidylcholine/ dipalmitoylphosphatidylcholine/cholesterol (DOPC/DPPC/Chol) and dioleoylphosphatidyl-choline/distearoylphosphatidylcholine/cholesterol (DOPC/DSPC/Chol) [1,2]. Substituting these values into theoretical calculations yields the energy penalty upon insertion of transmembrane proteins into Lo and Ld phases, and consequently the preferred partitioning in one of these domains. We discuss our findings for different geometric protein shapes.

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This work is supported by the Austrian Science Funds FWF, Project No. P24459.

Notes:

Poster 18:

Regulation of secreted phospholipase a2 expression and activation

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Secreted phospholipases A2 (sPLA2s) are enzymes that catalyze the hydrolysis of membrane phospholipids. They are differentially expressed in human tissues and are secreted from cells under various pathophysiological conditions, including cancer. The regulation of their expression, secretion and activity is however largely unknown. The aim of this study was to determine the expression and the mechanism of epigenetic and transcriptional regulation of sPLA2s in breast cancer, as well as to identify the proteases involved in the proteolytic maturation of human group X sPLA2 (hGX). Our results show that of the eleven human sPLA2 genes the expression of group IIA, III and X sPLA2s differs in tumour and normal tissue biopsies as well as in breast cancer cell lines of different molecular subtypes. Their transcription is differentially regulated by DNA methylation and by histone acetylation and, significantly, all three genes are silenced in aggressive triple negative cells due to both mechanisms. The transcription start site promoter region and upstream CpG islands, exclusive to the hGX sPLA2 gene, have variable roles in the regulation of sPLA2 expression in different breast cancer cells. Sp1 and several lipid transcription factors are most probably involved in the regulation of sPLA2 epigenetic silencing. The differential expression and epigenetic regulation of the three sPLA2s, along with the disparate effect of recombinant hGX sPLA2 on cell growth, suggests distinct roles for each enzyme in different breast cancer cell types.

Key words: DNA methylation, epigenetics, histone acetylation, secreted phospholipase A2, breast cancer

Notes:

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