799-Pos Board B678

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

Mathieu Noël, Aurélien Lorin, Marie-Éve Provencher,
Michéle Auger, Normand Voyer.
CREFSIP, CERMA, Université Laval, Québec, QC, Canada.

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

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

800-Pos Board B679

Biophysical Characterization And Membrane Interactions Of Peptides With Antimicrobial Potential

Aurélien Lorin[1], Mathieu Noël[1], Marie-Éve Provencher[2],
Mariza Gattuso[3], François Malouin[3], Normand Voyer[2],
Michéle Auger[1].
[1]CREFSIP, CERMA, Université Laval, Quebec, QC, Canada, [2]CREFSIP, Université Laval, Quebec, QC, Canada, [3]Biology Department, Université de Sherbrooke, Sherbrooke, QC, Canada.

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

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

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

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

References :
1) Y.R. Vandenburg, B.D. Smith, E. Biron, N. Voyer (2002) Chem Commun (Camb). 21:1694-1695.
2) M. Ouellet, F. Otis, N. Voyer, M. Auger (2006) Biochim Biophys Acta. 1758:1235-1244.

801-Pos Board B680

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

Jing He, Michelle Pate, Janet Hammer, **Jack Blazyk**.
Ohio University, Athens, OH, USA.

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

802-Pos Board B681

Molecular Mechanism of pEM-2 Activity

Amy Won, Anatoli Ianoul.
Carleton University, Ottawa, ON, Canada.

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

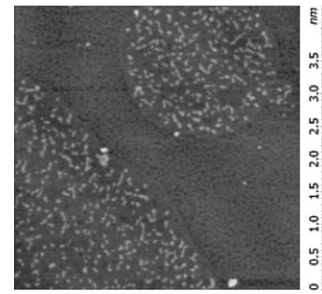

Figure 1. Contact mode AFM image (2x2 um) of a DPPG monolayer deposited at 30 mN/m in the presence of 400nM of pEM-2.

803-Pos Board B682

Deamidation Weakens Membrane Binding Properties of Antimicrobial Peptide Anoplin

Amy Won, Anatoli Ianoul.
Carleton University, Ottawa, ON, Canada.

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

804-Pos Board B683

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

Antje Pokorny, Erin M. Kilelee, Diana Wu, Paulo Almeida.
Univ. North Carolina Wilmington, Wilmington, NC, USA.

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

monounsaturated acyl chain. Except for the third series, peptide activity correlated with the first moment of the lateral pressure profile, which is a function of lipid acyl chain structure. In vesicles composed of asymmetric phosphatidylcholines, peptide binding and dye efflux are enhanced compared to symmetric, unsaturated lipids with similar pressure profiles. We attribute this to the entropically more favorable interaction of delta-lysin with partially saturated phospholipids. We find that lipid acyl chain structure has a major impact on the activity of delta-lysin and is likely to be an important factor contributing to the target specificity of amphipathic peptides.

805-Pos Board B684

Magainin 2 Revisited: a Test of the Quantitative Model for All-or-None Permeabilization of Phospholipid Vesicles

Paulo F. Almeida, Sonia M. Gregory, Antje Pokorny.
Univ North Carolina Wilmington, Wilmington, NC, USA.

The all-or-none kinetic model that we recently proposed for the antimicrobial peptide cecropin A is tested here for magainin 2. In mixtures of phosphatidylcholine (PC) /phosphatidylglycerol (PG) 50:50 and 70:30, release of contents from lipid vesicles occurs in an all-or-none fashion and the differences between PC/PG 50:50 and 70:30 can be ascribed mainly to differences in binding, which was determined independently and is about 20 times better for PC/PG 50:50 than to 70:30. Only one variable parameter, beta, corresponding to the ratio of the rates of pore opening to pore closing, is used to fit dye release kinetics from these two mixtures, for several peptide/lipid ratios ranging from 1:25 to 1:200. However, unlike for cecropin A where it stays almost constant, beta increases 5 times as the PG content of the vesicles increases from 30 to 50 percent. Thus magainin 2 is more sensitive to anionic lipid content than cecropin A. But overall, magainin follows the same all-or-none kinetic model as cecropin A in these lipid mixtures, with slightly different parameter values. When the PG content is reduced to 20 mole percent, dye release becomes very low; the mechanism appears to change, and is consistent with a graded kinetic model. We suggest that the peptide may be inducing formation of PG domains. In either mechanism, no peptide oligomerization occurs and magainin catalyzes dye release in proportion to its concentration on the membrane in a peptide state that we call a pore. We envision this structure as a chaotic or stochastic type of pore, involving both lipids and peptides, not a well-defined, peptide-lined channel.

806-Pos Board B685

Influence Of The Bilayer Composition On The Membrane-Disruption Effect Of Polybia-MP1, A Mastoparan Peptide With Antimicrobial And Leukemic Cell Selectivity

Marcia Perez dos Santos Cabrera[1], Manoel Arcisio-Miranda[2], Renata Gorjão[2], Natalia Bueno Leite[1], Bibiana Monson de Souza[3], Mario Sergio Palma[3], Rui Cury[2], João Ruggiero Neto[1], Joaquim Procopio[2].
[1]UNESP - São Paulo State University - IBILCE - Dept. of Physics, São José do Rio Preto - SP, Brazil, [2]USP - University of São Paulo - Biomedical Sciences Institute - Dept. of Physiology and Biophysics, São Paulo - SP, Brazil, [3]UNESP - São Paulo State University - CEIS - Institute of Biosciences, Rio Claro - SP, Brazil.

Unlike other mastoparans, Polybia-MP1 (IDWKKLLDAAKQIL), from the venom *Polybia paulista* (wasp), is highly selective for bacterial cells. By flow cytometry, we also found out this selective behavior: Polybia-MP1 promoted a decrease of 60 % cell viability at 25 μM in Jurkat (leukemic) cells, while it was not altered in primary human lymphocytes. The mechanism of selectivity was studied in the interaction with different bilayers. Ion channel-like activity was detected at 0.12 μM peptide concentration with anionic lipid membranes of azolectin, showing conductance in the range of 250 pS. On zwitterionic diphytanoylphosphatidylcholine-(DPhPC) it required 0.18 μM for the same conductance level. Further experiments with DPhPC bilayers containing 30% phophatidylserine or cardiolipin required higher peptide concentration to induce single channel events at slightly lower conductance levels. However, the presence of 20 mol% cholesterol in the mixture significantly reduced the ion channel-like activity, dropped the average conductance to around 120 pS and required 0.30 μM. On vesicles the activity of Polybia-MP1 also shows greater rate of leakage on the anionic over the zwitterionic, impaired by the presence of cholesterol; the lytic activity is characterized by a threshold peptide to lipid molar ratio that depends on the phospholipid composition. Preliminary results of changes in DPH anisotropy and acrylamide quenching of Trp fluorescence show a slight decrease in the anisotropy, and a significant quenching of the Trp fluorescence, indicating small influence on the lipid packing associated to preferential interaction with the lipid head group region. Results suggest that the selectivity of Polybia-MP1 is a consequence of a shallow interaction with zwitterionic bilayers, favored by the presence and position of negatively charged Asp residues, which is not possible for other mastoparan peptides.
Support: CAPES, CNPq, FAPESP

807-Pos Board B686

Effect of Antimicrobial Peptides from Australian Tree Frogs on Anionic Phospholipid Membranes

Frances Separovic[1], John D. Gehman[1], Tzong-Hsien Lee[2], John H. Bowie[3], Marie-Isabel Aguilar[4].
[1]University of Melbourne, Melbourne VIC, Australia, [2]Moansh University, Melbourne VIC, Australia, [3]University of Adelaide, Adelaide SA, Australia, [4]Monash University, Melbourne VIC, Australia.

Skin secretions of Australian tree frogs contain antimicrobial peptides that form part of the host defence mechanism against bacterial infection. The mode of action of these antibiotics is thought to be lysis of infectious organisms via cell membrane disruption, on the basis of vesicle-encapsulated dye leakage data [Ambroggio et al., (2005) Biophys. J. 89, 1874-1881]. A detailed understanding of the interaction of these peptides with bacterial membranes at a molecular level, however, is critical to their development as antibacterial therapeutics. We focus on four of these peptides, aurein 1.2, citropin 1.1, maculatin 1.1 and caerin 1.1, which exist as random coil in aqueous solution, but have α-helical secondary structure in membrane mimetic environments. In our earlier solid-state NMR studies, only neutral bilayers of the zwitterionic phospholipid dimyristoylphosphatidylcholine (DMPC) were used. Deuterated DMPC (d54-DMPC) was used to probe the effect of the peptides on the order of the lipid acyl chains and dynamics of the phospholipid head groups by deuterium and 31P NMR, respectively. We demonstrate several important differences when anionic phospholipid is included in model membranes. Peptide-membrane interactions were characterised using surface plasmon resonance (SPR) spectroscopy and solid-state NMR spectroscopy. Changes in phospholipid motions and membrane binding information provided additional insight into the action of these antimicrobial peptides. While this set of peptides have significant C- and N-terminal sequence homology, they vary in their mode of membrane interaction. The longer peptides caerin and maculatin exhibited properties that were consistent with transmembrane insertion while citropin and aurein demonstrated membrane disruptive mechanisms. Moreover, aurein was unique with greater perturbation of neutral versus anionic membranes. The results are consistent with a surface interaction for aurein 1.2 and pore formation rather than membrane lysis by the longer peptides.

808-Pos Board B687

The Alignment of Membrane-Active Peptides Depends on the Lipid Phase State as Viewed by solid state 19F-NMR

Sergii Afonin[1], Stephan L. Grage[1], Marco Ieronimo[2], Daniel Maisch[2], Parvesh Wadhwani[1], Pavel K. Mykhailiuk[3], Jesus Salgado[4], Igor V. Komarov[3], Anne S. Ulrich[2].
[1]Karlsruhe Institute of Technology/Institut für Biologische Grenzflächen-2, Karlsruhe, Germany, [2]Karlsruhe Institute of Technology/Institut für Organische Chemie, Karlsruhe, Germany, [3]National Taras Shevchenko University/Dept. Organic Chemistry, Kyiv, Ukraine, [4]Universitat de Valéncia/Dept. de Bioquímica i Biologia Molecular, Valencia, Spain.

Amphipathic membrane-active peptides (antimicrobial, hemolytic, cell-penetrating, fusogenic, etc.) achieve their functions by distinct interaction with lipid bilayers. Some typical structural modes are described in terms of models like the ''barrel stave'', ''toroidal pore'', ''carpet'' etc. These models are related to the alignment states of the peptides in the lipid bilayers (surface bound ''S-state'', inserted ''I-state'' or tilted ''T-state''), which can be readily characterized by solid state NMR. When determining such alignment, factors like peptide/lipid ratio, charge of the bilayer surface, thickness of the bilayer core, presence of cholesterol, and humidity are typically investigated. Yet, the lipid phase state as an explicit variable parameter has not received much attention so far. Here, we demonstrate that a change in the lipid phase can directly trigger the re-alignment of many peptides. Several representative examples are illustrated here: PGLa, PGLa/Magainin, gramicidin S, SAP and alamethicin. In macroscopically oriented DMPC bilayers, using highly-sensitive 19F-NMR we have monitored the changes between known alignment states of these peptides as a function of temperature, covering both the gel and liquid-crystalline states of DMPC. We show that for all peptides studied the alignment in the gel-state differs from the one in the liquid-crystalline bilayers and can be reversibly changed by passing through the lipid phase transition temperature. The relevance of these finding for the phase state of native biological membranes and interactions of membrane-active peptides with them will be discussed.