Membrane Active Peptides II

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Molecular Determinants of effective Pore Formation

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Although the alpha-toxin from *S. aureus* was the first pore-forming toxin identified, its mode of interaction with membranes is still not fully understood. The toxin forms heptameric pores on cellular and artificial membranes. The observation that artificial membranes are permeabelized by this toxin indicates that no protein receptor is mandatory. Efficient permeabilisation is only possible in presence of cholesterol and sphingomyelin, which could be interpreted as a preference of the toxin for raft-like structures. However, variation of lipid composition and determination of oligomer formation by pyrene-fluorescence reveals, that the toxin favors specifically sphingomyelin in combination with cholesterol and that the interaction is not raft-specific in a general sense. The mode of action seems also to differ from the primary binding of the toxin lysenin, which binds specifically to sphingomyelin.

Under certain conditions also non-lytic oligomers can be formed, for example at low temperatures or for certain mutants. The spectra obtained for pyrene-labelled mutants indicate that also lipid-composition might modulate the probability of lytic versus non-lytic pore formation, which might give a hint why for some cell non-lytic cells are found.

Employing both AFM and fluorescence microscopy we aim to determine the molecular basis for efficient pore formation by this toxin. We thank the DFG (SFB 490) for financial support, S.Bhakdi and A.Valeva for production of the toxin and helpful discussions, G. Gimpl for help with fluorescence microscopy and the MPI for Polymer Research for the possibility to use the AFM.

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Polycation Peptides Derived from the Primary Structure of *Bacillus thuringiensis* Cry11Bb Protoxin Permeabilize *Aedes aegypti* Midgut Mitochondria

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Cry toxins are highly active against dipteran insects, such as *Aedes aegypti*, but not against mammals, thus representing a base for development of environmentally safe insecticidal technologies. Earlier we demonstrated that BTM-P1, a 26 aa polycation peptide derived from domain I of the Cry11Bb protoxin, permeabilizes rat liver mitochondria and kill bacteria. In this work, we evaluated its capacity to permeabilize the inner membrane of *A. aegypti* larvae midgut mitochondria. The change in potential-dependent NAD(P)H fluorescence of the midgut homogenate was used as criteria of mitochondria permeabilization. When succinate was added, some increase in fluorescence intensity was observed, related to reverse electron transport. Addition of a known membrane protonophore FCCP caused a significant decrease in fluorescence intensity that can be completely recovered after subsequent addition of cyanide. A similar effect was observed by adding BTM-P1 or larger peptides BTM-P2 (37 aa) and BTM-P3 (60 aa) at concentrations of 1 uM, or even with the addition of natural hydrolysates of Cry11Bb obtained after treatment with larvae midgut homogenate. Cry11Bb trypsin hydrolysate was ineffective. Fast oxidation of endogenous NAD(P)H, mitochondrial swelling and inner membrane potential drop were observed after addition of the peptides to isolated rat liver mitochondria. We suggest that, in addition to the binding of Cry toxins to the corresponding midgut receptors, the subsequent natural proteolysis of domain I might produce various polycation peptides that cause permeabilization of the plasma and mitochondrial membranes by a potential-dependent manner that should be favored by the lumen positive transepithelial potential of the posterior midgut. This work was supported by Colciencias grants #2213-12-17833 and #111840820380.

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Phosphatidylserine Selective Peptides As Novel Anti-cancer Agents

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Antimicrobial peptides have gained interest as potential anti-cancer agents. Phosphatidylserine (PS), which normally resides in the inner leaflet of the plasma membrane, can move to the outer leaflet and act as a surface marker of cancer cells. The surface exposure of negatively charged PS on prostate tumour cells makes these cells susceptible to killing by cationic membranolytic peptides such as NK-2 [1]. Shai et al. [2] have also shown the inhibition of tumor growth in human prostate xenografts by host defense like lytic peptides. The aim of this study is to develop short peptide sequences acting selectively towards PS exposed on cancer cells. As a prerequisite it is necessary to analyze the lipid composition of prostate cancer cell - and non cancer cell plasma membranes. Further as a basis for peptide activity studies the biophysical characteristics of cancer cell membranes and healthy counterparts with respect to lipid composition were determined by DSC and X-ray studies with liposomal mimics. Fluorescence spectroscopy was applied to test the release of marker molecules from liposomes, which revealed that some NK-2 derived peptides have a high affinity towards PS.

Results of the in-vitro testing of optimized peptides on prostate cancer cell lines together with biophysical data help us to shed light on the future evaluation of their usage as anticancer therapeutics.

[1] Schröder-Borm H. et al., FEBS Lett. 579 (2005) 6128-61.
[2] Shai Y. et al., Cancer Research 2006; 66 (10) May 15.

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Human Erythrocytes And Mononuclear Leukocytes Are Capable Of Concentrating HIV-1 Fusion Inhibitor Peptides In Their Membranes

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Following the successful approval of the first HIV-1 fusion inhibitor, enfuvirtide (formerly T-20), T-1249 was developed to be one of the next generation drug of this type. Previous studies, based on tryptophan intrinsic fluorescence, showed that these peptides interact with membrane model systems (large unilamellar vesicles) of different lipid compositions. Studies with human blood cell membranes were necessary to further establish the role of membranes on these peptides mode of action. An experimental strategy was applied taking into account the membrane dipole potential as measured by the potential sensitive fluorescent probe di-8-ANEPPS. Successful labelling was performed with erythrocytes and peripheral blood mononuclear cells (PBMC) isolated from human blood samples.

For erythrocytes, membrane bound di-8-ANEPPS excitation spectra were blue shifted, indicating a decrease in the dipole potential due to peptide-membrane interactions. Accordingly, a decrease in the probe fluorescence excitation ratio (a measure of the spectral shift) dependent of peptide concentration was observed. The quantitative analysis of these variations indicated that T-1249 had the higher affinity towards erythrocyte membrane. This is in agreement with the previously known adsorption of this peptide on cholesterol-rich membrane domains.

Preliminary results show that the behaviour is similar in the case of PBMC, with a decrease in dipole potential that is more pronounced for T-1249. As there is strong suggestion that HIV also associates with erythrocytes *in vivo*, the peptide concentration effect of the erythrocytes and lymphocyte membranes can correlate with the stronger efficacy of T-1249.

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Membrane Interaction of N-Terminal Peptides of Annexin A1

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Annexin A1 is a member of a family of calcium-dependent membrane-binding proteins. This protein is known for its anti-inflammatory effect and for its ability to aggregate phospholipid membranes. A N-terminal annexin A1 peptide activates and desensitizes the human N-formyl peptide receptor (FPR), a member of G-protein coupled receptors that is a key modulator of chemotaxis directing granulocytes toward sites of bacterial infections. The N-terminal domain of annexin A1 mainly seems to be responsible for the biological activities of this protein. So we are interested in the (Ca^{2+}-independent) membrane binding activities and structural information of the phospholipid membrane interaction of N-terminal peptides of annexin A1.

We investigated the membrane interaction of synthesized N-terminal peptides of annexin A1 (residues Ac2-26 and 1-40) with artificial vesicles of different lipid compositions (mainly phosphatidylserine and phosphatidylcholine) and under varying buffer conditions (ionic strength, pH of the solution) by using fluorescence techniques. For these measurements we used the A1NT peptide labelled at 4 different positions with a dansyl fluorophore. From the experimentally measured binding curves the Gibbs free energy for the peptide transfer from aqueous solution to the lipid membrane was calculated. The effective charge of the peptide depends on the pH value of the buffer and is about half of its theoretical net charge. Fluorescence correlation spectroscopy measurements were done with TAMRA labelled A1NT peptide and giant unilamellar vesicles. The binding of fluorescently labelled peptides to micro-domains (lipid rafts) in differently compounded giant vesicles we observed using confocal laser scanning microscopy. Structural information of membrane bound peptide we reached by polarized infrared spectroscopy (ATR-FTIR). Further, the position of A1NT α-helix in the membrane was estimated from the intrinsic

tyrosine fluorescence, from quenching experiments with spin labelled phospholipids using A1NT Trp.

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Interaction Of Human Islet Amyloid Poly Peptide With Phospholipid Membrane Vesicles

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Amylin, also known as Human Islet Amyloid Polypeptide (hIAPP), is a 37-residue peptide, suspected to play a major role in the malfunction of insulin secretion in diabetes mellitus type II. Co-secreted with insulin in the beta cells, hIAPP, in higher rates destroys the barrier function of the beta-cells, leading to a failure in insulin production. Because of its amyloidogenity, aggregates of fibrils can be observed in the islands of Langerhans due to its overexpression. We studied the physico chemical properties of hIAPP by observing changes in its structure depending on time and the surrounding media using MALDI-TOF-MS, ATR FT-IR- and fluorescence spectroscopy. In water, hIAPP fibrils grow slowly, after a 37°C incubation for 24 hours some alpha-helices are twisted, and after two weeks, no random coil is detected anymore. We determined membrane binding of dansyl-labeled hIAPP to phosphatidylserine (PS)/ phosphatidylcholine (PC) membranes. Additionally, using confocal laser scanning microscopy the binding of TAMRA labelled hIAPP to giant unilamellar vesicles could be observed. At physiological pH, hIAPP is positively charged and thus negative charges at the phospholipid membrane surface accelerate the process of peptide folding. Being random coil as initial state, a mixture of antiparallel beta-sheets and alpha-helices emerges in time. In the presence of negatively charged PS/PC membranes, hIAPP aggregates can be seen within a few minutes after titration. To understand the process of penetration into cells, we performed leakage measurements of carboxyfluoresceine (CF) filled phospholipid large unilamellar vesicles by means of fluorescence spectroscopy. Titration of hIAPP to CF filled PS/PC liposomes showed different results concerning equilibrium time and maximal extent of leakage depending on the age and preparation of the peptide. In particular the composition of the vesicles seems to determine their stability in the presence of hIAPP.

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Single Particle Analysis of Liposome Leakage Induced by Islet Amyloid Polypeptide

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Islet Amyloid Polypeptide (IAPP) is a 37-residue peptide hormone co-secreated with insulin from pancreatic β-cells. In patients suffering from type-2 diabetes mellitus (T2D), IAPP forms amyloid fibers in the pancreas, which are associated with cell death and the progression of the disease. A possible mechanism of cytotoxicity in T2D is the permeablizing of membranes by oligomeric IAPP, followed by leakage of ions or other molecules. We are examining the IAPP-induced leakage of individual liposomes through the use of single particle methods. Individual fluorescently labeled liposomes are measured post-IAPP exposure through the use of single particle burst analysis to determine the distribution of leakage states. By determining the role of individual residues, solution conditions, and lipid composition in modulating leakage insights are made into the mechanism of oligomer-mediated membrane leakage.

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Amyloid Oligomers Alter The Conductance Of The Gramicidin Channel

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Amyloid oligomers alter the conductance of the gramicidin channel.

Our previous data suggest that Aβ does not itself contribute a new intrinsic conductance such as ion channel to the membrane, but it does seem to alter its physical properties, specifically increasing the apparent dielectric constant of hydrocarbon region. This effect could in turn affect the properties of membrane ion channels.

In order to test this notion we compared the effects of amyloid oligomers on the single channel conductance of gramicidin in 2 M NaCl and CsCl. Amyloid oligomers increase the single channel conductance in NaCl from 13 to 16 pS, but the situation in CsCl is more complicated. In CsCl, the single channel conductance histogram shows two peaks, one with a conductance essentially the same as control (42 pS) and one with a conductance significantly less than control (28 pS). In terms of a simple three barrier two site model such as that used by Barnett et al., 1986 this suggests that amyloid oligomers lower the energies of both Cs and Na ions in the gramicidin channel, but at different critical locations relative to the barrier profile. For Na^+, amyloid oligomers lower the principal central barrier and thus increase the translocation rate of Na^+ at a given voltage. For Cs^+, amyloid oligomers act as if they lower the energy of the Cs ion in the channel, but in such a way as to increase the depth of one or both of the two wells in the barrier profile.

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The Insulin-sensitizers Troglitazone And Rosiglitazone Alter Lipid Bilayer Properties

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Thiazolidinediones are widely used to treat hyperglycemia in patients suffering from type 2 diabetes. Three thiazolinediones - troglitazone (Resulin), rosiglitazone (Avandia), and pioglitazone (Actos) - have been marketed; troglitazone was subsequently withdrawn due to hepatotoxicity. The thiazolidinediones are selective peroxizome-proliferator receptor gamma (PPARγ) agonists and they increase insulin sensitivity. They also have been found to have anti-oxidant, anti-inflammatory, anti-atherosclerotic and cardiovascular effects, but PPARγ activation alone does not account for all their actions. All three derivatives, with troglitazone being the most potent, modulate L-type calcium and delayed-rectifier potassium Kv1.3 channels by a seemingly PPARγ-independent mechanism. This could result from the adsorption of amphiphilic molecules to the membrane, which can alter bilayer properties such as thickness, intrinsic curvature and elastic moduli, and thus membrane protein function. We therefore set out to determine whether the amphiphilic troglitazone and rosiglitazone alter lipid bilayer properties. Using gramicidin channels as probes, where we monitor the changes in channel lifetime and rate of appearance, we tested and compared the effects of troglitazone and rosiglitazone on channels of different lengths in DOPC bilayers. Troglitazone or rosiglitazone did not alter gramicidin channel conductances, suggesting that direct interactions are not involved. In contrast, the lifetimes of both channels increased with similar relative changes for both the shorter and the longer channels. Consistent with their effects on calcium and potassium channels troglitazone is more potent than rosiglitazone. Our results show that both troglitazone and rosiglitazone affect bulk membrane properties at the concentrations where they modulate other ion channels.

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The Antimicrobial Peptide Gramicidin S Permeabilizes Phospholipid Bilayer Membranes Without Forming Discrete Ion Channels

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We examined the permeabilization of lipid bilayers by the β-sheet, cyclic antimicrobial decapeptide gramicidin S (GS) in phospholipid bilayers formed either by mixtures of zwitterionic diphytanoylphosphatidylcholine and anionic diphytanoylphosphatidylglycerol or by single zwitterionic unsaturated phosphatidylcholines having various hydrocarbon chain lengths, with and without cholesterol. In the zwitterionic bilayers formed by the phosphatidylcholines, without or with cholesterol, the peptide concentrations and membrane potentials required to initiate membrane permeabilization vary little as function of bilayer thickness and cholesterol content. In all the systems tested, the GS-induced transient ion conductance events exhibit a broad range of conductances, which are little affected by the bilayer composition or thickness. In the zwitterionic phosphatidylcholine bilayers, the effect of GS does not depend on the polarity of the transmembrane potential; however, in bilayers formed from mixtures of phosphatidylcholines and anionic phospholipids, the polarity of the transmembrane potential becomes important, with the GS-induced conductance events being much more frequent when the GS-containing solution is positive relative to the GS-free solution. Overall, these results suggest that GS does not form discrete, well-defined, channel-like structures in phospholipid bilayers, but rather induces a wide variety of transient, differently sized defects which serve to compromise the bilayer barrier properties for small electrolytes.

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The Superstructure of an Antimicrobial Peptide, Alamethicin, in Lipid Membranes

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In this work we investigate the effect of membrane hydration and hydrophobic mismatch on the Alm channel superstructure in an oriented multilayer sample by x-ray scattering. Wide angle x-ray scattering (WAXS) near 1.4 $Å^{-1}$ indicates that the lipid chain region is not much perturbed by the incorporation of up to 10 mole percent Alm. Low angle x-ray scattering (LAXS) indicates that when the sample is very dry, which promotes interactions between neighboring