Membrane Active Peptides II

809-Pos Board B688
Molecular Determinants of effective Pore Formation
Antje Brack, Markus Schwiering, Christian Beck, Max Koistinen, Heinz Decker, **Nadja Hellmann**.
Institute for Molecular Biophysics, Mainz, Germany.
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.

810-Pos Board B689
Polycation Peptides Derived from the Primary Structure of *Bacillus thuringiensis* Cry11Bb Protoxin Permeabilize *Aedes aegypti* Midgut Mitochondria
Victor V. Lemeshko[1], Gabriela Jaramillo[2], Sergio Orduz[1].
[1]Universidad Nacional de Colombia, Sede Medellin, Colombia,
[2]Corporación para Investigaciones Biológicas, Medellin, Colombia.
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.

811-Pos Board B690
Phosphatidylserine Selective Peptides As Novel Anti-cancer Agents
Yasemin Manavbasi[1], Dagmar Zweytick[1], Regine Willumeit[2], Karl Lohner[1].
[1]Austrian Academy of Sciences, Graz, Austria, [2]GKKS, Geesthacht, Germany.
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.
Acknowledgement: Marie Curie Actions ''Biocontrol''.

812-Pos Board B691
Human Erythrocytes And Mononuclear Leukocytes Are Capable Of Concentrating HIV-1 Fusion Inhibitor Peptides In Their Membranes
Pedro M. Matos[1,2], Miguel A.R.B. Castanho[1,2], Nuno C. Santos[1,2].
[1]Instituto de Medicina Molecular, Lisboa, Portugal, [2]Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal.
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.

813-Pos Board B692
Membrane Interaction of N-Terminal Peptides of Annexin A1
Heiko Weigelt, **Olaf Zschörnig**.
University of Leipzig, Leipzig, Germany.
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