# Peptide & Toxin Ion Channels

**2748-Pos Board B718**

**Reconstitution Of Tonb-dependent Transporters In Planar Lipid Bilayers**

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Micronutrients such as siderophore-bound iron and vitamin B12 cross the outer membrane of gram-negative bacteria through a group of 22-stranded β-barrel proteins. They share the unusual feature that their N-terminal end inserts from the periplasmic side into the β-barrel and plugs the lumen. Transport results from energy-driven movement of TonB protein, which either pulls the plug out of the barrel or causes it to rearrange within the barrel to open a space wide enough for siderophores to diffuse through.

Attempts to reconstitute these plugged channels in an ion-conducting state in lipid bilayer membranes have so far been unsuccessful. Recently, however, we have discovered that if the cis solution contains 4 M urea, then macroscopic conductances and single channel events could be observed. These results were obtained with FhuA, Cir and BtuB, and in the case of the former two, the channels were closed by removing the 4 M urea. The basic native structure of these proteins appears to be preserved by our reconstitution procedure. With FhuA, for example, addition of ferrichrome (its siderophore) to the trans side reversibly inhibits 4 M urea-induced channel opening and blocks the channels with a Kd of ~0.5 nM, the same as that found biologically. In the case of Cir, as another example, addition of colicin Ia (the microbial toxin that targets Cir) to the trans side prevents 4 M urea-induced channel opening. Our working hypothesis is that the 4 M urea reversibly denatures the plug, causing it to either rearrange itself or to come completely out of the barrel, thereby providing a conduction pathway. Whether the 4 M urea action on the plug mimics to some extent that of TonB remains for future investigation.

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**Ion Channel Activity of Amyloid-beta Peptides in Artificial Bilayer Membranes: a Comparison of Different Preparation Techniques**

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Alzheimer's disease (AD), an ultimately fatal neurodegenerative disorder, is characterized by the presence of plaques containing fibrillar aggregates of amyloid-beta (Aβ) peptides. These peptides, with 39-43 amino acids, especially Aβ(1-40) and Aβ(1-42), are the major components of plaques formed in the brain of patients with AD. Both peptides aggregate rapidly in aqueous solution to form Aβ oligomers as well as Aβ fibrils. An increasing number of evidence suggests that Aβ especially in non fibrillar forms interacts to cell membrane by forming an ion channel or pores through the membrane. One approach to study the mechanism of Aβ is to examine the ion channel activity of Aβ on lipid membrane. A challenge of studying ion channel of Aβ is the difficulty in obtaining the ion channel activity consistently. This study summarizes the 4 different methods of preparation of Aβ(1-40) and Aβ(1-42) which can form ion channel activity on the artificial membrane. We also provide the information on characterization of structural properties, size and kinetics of aggregation of Aβ corresponding to each method of preparation. Our studies may be useful for those who are interested in ion channel approach of Aβ as well as other peptides which share common characteristics in forming ion channel and involve with other amyloid diseases.

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**Pore Forming Properties Of Antimicrobial Peptides In Different Natural Lipid Environment**

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The biophysical characteristics and the pore formation dynamics of synthetic, naturally occurring peptides forming membrane-spanning channels, were investigated by using cells having high membrane resistance recorded in whole-cell configuration. The peptides were applied to (and removed from) the recorded cells in $\lesssim 50$ ms that were: isolated photoreceptor rod outer segments (OS) of low vertebrates, their red blood cells (RBC), and human RBC. The OS membrane resistance was $\geq 5$ GΩ once the OS main endogenous conductance was blocked with light; the RBC had lower resistances but high enough to allow the detection of the exogenous current produced by the peptides up to single channel resolution. Surprisingly, the synthetic, naturally occurring alamethicin F50/5 and selected analogs produced current amplitudes once inserted in the OS at least twice the ones in RBC: therefore, the lack of hemolysis does not ensure the lack of side-effects of an antimicrobial peptide. Once in the RBC membrane, the alameticines produced pores whose voltage-dependency and single channel properties were similar to the ones recorded in OS, showing that the pore forms only as a barrel-stave, independently by the lipid environment. Other modes of pore formation were investigated with cecropin-melittin hybrid peptide (Acetyl-KWKLFKKIGAVLKVL-$CONH_2$), that produced reversible membrane permeabilization at concentrations up to 10 μM. This reversibility at such high concentrations is not expected in the case of a carpet model of pore formation, where micellation should be severe. At difference with alamethicines, membrane permeabilization was voltage-independent, repetitive peptide application caused the progressive increase of the steady-state current amplitude, and no discernible single-channel events were detected at low peptide concentrations. Collectively, these results indicate that cecropin-melittin hybrid peptide permeabilize the membrane according to a toroidal model of pore formation (instead of barrel-stave or carpet one).

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**Rapid Topology Determination of Membrane Proteins: Pore-Forming Mechanism of Bt toxin Cry1Aa**

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While crystal structures provide a structure with atomic resolution, it is only limitedly applicable to membrane toxins. The reason is that the mechanism of the toxins is based on a complete refolding of the protein ones it incorporates into the membrane. Cry1Aa, a toxin of *Bacillus thuringiensis*, is a widely used biological pesticide. It has been shown that the toxin consists of three structural distinct domains. While domains II and III are involved in receptor binding and host specificity, domain I forms cation-selective pores in bilayer membranes. The present study aims to investigate the topology of the toxin at the membrane previous to the pore formation and the structural determinants of the pore forming mechanism of Bt toxin. Previous models predicted that the toxin anneals to the surface of the membrane before triggering the pore formation by inserting the α-helices 4-5 through the membrane, while the other helices are thought to remain on the external leaflet *(Vachon et al., 2003)*. We labelled the toxin by site-directed fluorescence labelling and inserted it into horizontal planar lipid bilayer. We performed FRET measurements between these distinct positions in the toxin and the bilayer. This way, we could determine the location of the residues with respect to the centerline of the membrane and create a topology map of the protein. In contrast to the previous model, we found that most of domain I translocates through the membrane upon insertion and accumulates in the inner leaflet. Preliminary data suggest that for pore formation the helices 3 and 4 translocate through the membrane from the inner to the outer leaflet. This is consitent with helix 4 being the pore-lining helix as suggested by electrophysiological data.

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**Protonophoric Activity Of Gramicidin A Modified By Charged Aminoacids At Its N-terminus**

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Introducing a charged group near the N-terminus of gramicidin A (gA) is supposed to suppress its ability to form ion channels by restricting its head-to-head dimerisation. However, des-formyl-gramicidin was earlier found to exhibit significant protonophoric activity both in artificial and mitochondrial membranes. The present study deals with the activity of [Lys1]gA, [Lys3]gA, [Glu1]gA and [Glu3]gA in model membrane systems (planar lipid bilayers and liposomes) and mitochondria. All of the peptides induced proton conductivity in liposomes with almost the same potency, however, cationic derivatives were also able to cause non-specific leakage from liposomes. Measurements of electrical current through a planar lipid membrane at 100 mM HCl showed the formation of gA-like ion channels. However, effective concentrations of cationic peptides were by two orders of magnitude higher than those of anionic peptides. [Glu1]gA displayed considerably more pronounced effect on mitochondrial membrane potential compared to other peptides. A study of the properties of hybrid channels composed of cationic and anionic peptides is in progress.