ethanolamine (PE18:1/16:0) and phosphatidyl serine (PS18:0/18:1). Since this membrane is thicker than the estimated length of tolaasin channel, mismatch in thickness may make the channel unstable. Phospholipids composed of medium or short-chain fatty acids may be helpful to the stability of tolaasin channel by making the membrane thinner. When phosphatidyl ethanolamines made with decanoic acids (capric acid, DDPE), myristic acids (DMPE), and stearic acids (DSPE) were added, DDPE (200 nM) facilitated tolaasin-induced hemolysis. When the concentration of DDPE was adjusted from 0.2-200 nM, the hemolysis was stimulated at the concentrations above 2 nM. Ks value of DDPE effect was obtained at 6 μM DDPE. When the preincubation effect of tolaasin and DDPE was measured, binding of tolaasin to DDPE was completed within 5 min. In the lipid bilayer recording, the addition of DDPE increased tolaasin channel activity by increasing open probability. Therefore, tolaasin molecules make more stable channels with phospholipids composed of medium-chain fatty acids.

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Competition Effect of DDPE and Zn^{2+} on the Hemolysis Induced by Tolaasin, a Pore-Forming Peptide Toxin

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Tolaasin is an antimicrobial peptide produced by *Pseudomonas tolaasii* and causes a brown blotch disease by disrupting membrane structures of cultivated mushrooms. The pore-formation of tolaasin was demonstrated in an artificial lipid bilayer. The length of tolaasin channel is not enough to penetrate through the membrane. The additions of phospholipids composed of two medium-chain decanoic acids (DDPE) facilitated tolaasin-induced cytotoxicity. When tolaasin was added to RBCs, its hemolytic activity was increased by the addition of DDPE at the concentrations above 2 nM. Although various tolaasin inhibitors have been isolated and characterized, no activators are identified up to date. Therefore, working mechanism of DDPE will tell us about very useful information how tolaasin works on the membrane. In the presence of DDPE, the complete hemolysis occurred very fast, within 5 min, compared to the control experiment of 25 min. Competition effect of Zn^{2+}, a potent tolaasin inhibitor, and DDPE on tolaasin-induced hemolysis was investigated. When Zn^{2+} and DDPE were added simultaneously, the stimulatory effects of DDPE observed at low concentrations of Zn^{2+}. Zn^{2+} at 0.5 mM inhibited the tolaasin-induced hemolysis by 70%; however, it was reduced to 30% in the presence of DDPE at 1 μM. At the concentrations above 1 mM, Zn^{2+} completely blocked the tolaasin activity and no effect of DDPE was measured. In these experiments, the effect on the tolaasin-induced hemolytic activity was dominated by Zn^{2+} rather than DDPE at Zn^{2+} concentrations above 1 mM. Mixed effect of these two modulators was observed at 0.5 mM Zn^{2+}. Based on these results, we suggest that DDPE stabilizes the tolaasin channel but it does not inhibit the binding of Zn^{2+}, representing their binding sites or working mechanisms are different.

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Protein Translocation through Mitochondria Channel: Peptide Interactions with TOM40 Channel

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TOM is high molecular mass protein complex that facilitates the transfer of nearly all mitochondrial preproteins across outer mitochondrial membrane. Preproteins bound by different receptor subunits travel via a pore formed by a specific subunit that constitutes an ion-conducting channel into mitochondria. High resolution ion conductance measurements through mitochondrial TOM40 channel in the presence of peptide revealed binding kinetics. More specifically, we have investigated the voltage dependence of the membrane transport of the peptide pF1β through single TOM40 channel. It is shown that association rate $k$on and dissociation rate k_{off} strongly depends on the applied transmembrane voltage and kinetic constants increase with increase in the applied voltage. This model involves a binding site inside the channel and attractive interactions between the peptide and binding site in the channel facilitates the peptide translocation at increasing voltage. Our analysis of the data provides a full quantitative description of all the relevant thermodynamic, kinetic and electric parameters including a detailed formulation of the peptide partition through the channel at single molecule level.

Reference

Romero-Ruiz M, Mahendran KR, Eckert R, Winterhalter M, Nussberger S. Interactions of mitochondrial presequence peptides with the mitochondrial outer membrane preprotein translocase TOM. *Biophys J.* 99 (2010) 774-81.

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Electrical Aspects of Membrane Permeabilization by New Polycationic Peptides Derived from the Cry11Bb Protoxin

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The permeabilization of mitochondrial and plasma membranes by synthetic polycationic peptides derived from the Cry11Bb protoxin was studied. The peptides were designed with the aim to further study of their antimicrobial and anticancer activities. It was observed that the membrane permeabilizing activity of these polycationic peptides strongly increased by the transmembrane potential (minus inside). This phenomenon was confirmed by the study of the artificial planar membrane permeabilization: applying 50 mV to the planar membrane (minus to the trans side) during 30 sec induced time-dependent increase in the transmembrane current in the presence of a peptide added to the cis side, while subsequent application of the opposite potential caused its decrease. We also observed that the activation of the cell suicide mechanism, which partially revealed in phosphatidylserine exposure at the cell surface, significantly increased the plasma membrane permeabilization by polycationic peptides. Some peptide topology characteristics, such as the value and the orientation of the electrical dipole moment(s) interacting with the membrane dipole potential seem to also be important factors influencing the membrane-permeabilizing activity of polycationic peptides. In general, our data are consistent with the concept that various electrical properties of biomembranes (the transmembrane potential, membrane dipole potential and the surface charge) might explain at least partially certain selectivity of antimicrobial and anticancer activities of many natural and synthetic polycationic peptides. (Financial support: Colciencias (Colombia) research grant #111840820380).

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Helix N-Cap Asp are the pH Trigger for Colicin a Membrane Insertion

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Colicins are antibacterial toxins produced by *Escherichia coli* cells to kill closely related competing bacteria. They are large proteins (>40kDa) composed of three domains. The N terminal and central domains are required to cross the gram negative outer membrane whilst the C-terminal domain carries a toxic activity such as a nuclease or pore-forming function. The pore forming domains are all homologous, ten helix bundles with a buried hydrophobic hairpin which is exposed in the membrane bound state. As a result, unfolding of the domain is required for function and, for some colicins such as N, this is a significant barrier to in vitro study. Two colicins, A and B, show significantly enhanced membrane insertion at low pH and this has been correlated with the formation of an acidic molten globule. Furthermore colicin A requires acidic lipids in the inner membrane of target cells whilst colicin N, which shows no pH sensitivity, does not. The mechanism for this pH sensitivity has been unclear. Here we show that helix N-Cap residues are the critical pH switch. At several sites in Colicin A, surface Asp residues replace the Asn found in colicin N. Surprisingly mutation of these surface Asp to Ala leads to a molten globule phenotype whereas replacement by Asn stabilises the domain. HSQC NMR shows that the effects of the Ala mutations are not localised. Examination of the structure at each site shows that each critical Asp is a helix N-capping residue. Protonation of each Asp destabilises the colicin and allows membrane insertion. This not only solves the riddle of colicin A pH dependence but also reveals a generic method for pH regulation of protein stability.

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On Channel Activity of Synthetic Peptides Derived from Severe and Acute Respiratory Syndrome Coronavirus (SARS-CoV) E Protein

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Members of the *Coronaviridae* family are enveloped viruses causing in humans from common colds to acute respiratory syndrome and, in animals, a variety of lethal diseases of economical relevance. The envelope (E) protein from coronaviruses (CoVs) is a small polypeptide that modulates coronavirus morphogenesis, tropism and virulence [1]. SARS-CoV E protein forms an amphipatic alpha-helix that expands the viral membrane once and displays