Abstract (English)

This work is based on five publications studying mostly adenylate cyclase toxin (CyaA) from *Bordetella pertussis* and its interaction with biological membranes. CyaA permeabilizes cell membranes by forming small cation-selective pores and subverts cellular signaling by delivering an adenylate cyclase (AC) enzyme that converts ATP to cAMP into host cells.

First study clarifies the membrane disruption mechanisms of CyaA and another bacterial RTX toxin; α-hemolysin (HlyA) from *Escherichia coli*. For this purpose, we employed a fluorescence requenching method using liposomes as target membranes. We showed that both toxins induced a graded leakage of liposome content with different ion selectivities (Fišer a Konopásek 2009).

Both AC delivery and pore formation were previously shown to involve a predicted amphipathic α-helix(502-522). In the second publication we investigated another predicted transmembrane α-helix(565-591) that comprises a Glu(570) and Glu(581) pair. We examined the roles of these glutamates in the activity of CyaA, mostly on planar lipid membranes end erythrocytes. Negative charge at position 570, but not at position 581, was found to be essential for cation selectivity of the pore, suggesting a role of Glu(570) in ion filtering close to pore mouth. The pairs of glutamate residues in the predicted transmembrane segments of CyaA appear to play a key functional role in membrane translocation and pore-forming activities of CyaA (Basler et al. 2007).

In the next work we describe a new activity of CyaA that yields elevation of cytosolic calcium concentration ($[Ca^{2+}]_i$) in target cells. The CyaA toxin during the bacterial infection targets primarily phagocytes expressing the $\alpha_M\beta_2$ integrin (CD11b/CD18). The CyaA-mediated $[Ca^{2+}]_i$ increase in $CD11b^+$ J774A.1 monocytes was inhibited by extracellular La^{3+} ions but not by any specific inhibitor of cellular channels, suggesting that influx of Ca^{2+} into cells was not because of receptor signaling or opening of conventional calcium channels. The translocating AC polypeptide itself appears to participate in formation of a novel type of unexpected membrane path for calcium ions (Fišer et al. 2007).

We show that penetration of the AC domain across cell membrane proceeds in two steps. It starts by membrane insertion of a toxin 'translocation intermediate', that permeabilizes cells for influx of extracellular Ca^{2+} and thus activates calpain-mediated cleavage of the talin tether. Recruitment of the integrin-CyaA complex into lipid rafts follows and the cholesterol-rich lipid environment promotes full translocation of the AC domain across cell membrane (Bumba et al. 2010).

The ability of CyaA to promote influx of Ca^{2+} into cells dictates the path and kinetics of subsequent endocytic removal of CyaA toxoids from phagocyte membrane. Clathrin-dependent endocytosis and transit of CyaA through transferrin-containing early recycling vesicles was observed with the toxoid capable to promote Ca^{2+} influx and to associate with membrane lipid rafts. This uptake path allowed delivery, processing and presentation of toxoid-fused antigens on MHC class II molecules. In turn, a mutated toxoid, unable to mediate Ca^{2+} influx into cells, was rapidly taken-up from cellular membrane by a clathrin-independent macropinocytic mechanism (Fišer et al. 2011).

Keywords

Bordetella, CyaA , protein translocation, membrane channels, planar lipid membranes, calcium signalling, endocytosis, liposome disruption, macrophage, Fura-2