Abstract

Adenylate cyclase toxin (CyaA) is one of the major virulence factors of bacterium *Bordetella pertussis*, which is a causative agent of whooping cough. CyaA belongs to the family of RTX toxin-hemolysins. The toxin targets primarily cells expressing integrin receptor CD11b/CD18 but it can also penetrate cells lacking this receptor. CyaA acts on host cells by two independent activities. One is formation of small cation-selective channels, which can lead to colloid osmotic lysis of target cells. The second is disruption of cell signaling through the translocation of the adenylate cyclase (AC) domain to host cell cytosol, which leads to the conversion of ATP into cyclic AMP.

It was recently shown that cholesterol affects endocytosis of CyaA. CyaA translocates it´s AC domain after relocation of CyaA molecule to the cholesterol-rich lipid raft (Bumba et al. 2010). In this work I examined the effect of cholesterol on channel-forming activity and selectivity of ion channels created by CyaA. For measurements I used artificial membranes enriched with cholesterol.

CyaA channels are voltage-dependent. The positive membrane potential on the side of toxin is rquired for incorporation of CyaA molecule into cell membrane. I tried to find out whether the value of voltage has effect on channels opening time.

Calcium ions are essential for toxic activity of CyaA. CyaA requires calcium ions for formation of hemolytic channels and for folding of RTX domain, delivery of its AC domain into cells. The second objective of this study was to determine whether calcium ions pass through the CyaA channel.

The last aim of this work was to determine the size of CyaA channels. The estimated inner diameter ranges from 0.6 to 0.8 nm, so far. I used nonelectrolytes, uncharged substances, for the experiments in this work. Based on changes in the conductivity of CyaA channels it can be considered how large molecules can pass through pore.

All experiments were carried out on apparatus for measuremets on black lipid membranes.