

The adenylate cyclase toxin (CyaA) of *Bordetella pertussis* is a 1706-residue protein comprising an amino-terminal adenylate cyclase (AC) domain and a carboxy-terminal Repeat-in-Toxin (RTX) domain. The RTX domain is a hallmark of the family of RTX proteins, which are secreted from the cytosol of Gram-negative bacteria to the cell environment through the Type I Secretion System (T1SS). The RTX domain of CyaA consists of five blocks of RTX nonapeptide repeats with a consensus sequence X-(L/I/V)-X-G-G-X-G-X-D. The aim of this work was to determine the role of the RTX domain in biological activities of CyaA and its role in the secretion of the toxin molecule from *Bordetella pertussis*. Systematic deletion analysis revealed that none of the prepared CyaA constructs was able to translocate its AC domain across the cytoplasmic membrane of host cells and make pores in target membranes. Moreover, deletion of individual RTX repeat blocks resulted in a very low efficacy of secretion of CyaA mutants into cell exterior. These data suggested that structural integrity of the RTX domain of CyaA is essential not only for cytotoxic activities of the toxin molecule but also for its secretion through the T1SS.