ABSTRACT

Adenylate cyclase (CyaA, ACT) toxin is one of the major virulence factors of *Bordetella pertussis*. Although CyaA binds to many types of membranes, it is assumed that the integrin CD11b/CD18 is its receptor which is expressed on the surface of myeloid cells. CyaA belongs to the family of RTX toxin-hemolysins. CyaA acts on the host cells by two independent activities. One of them is the conversion of ATP to cyclic AMP, which is catalyzed by adenylate cyclase (AC) domain after its translocation into the cytosol of the host cell, which leads to the entry of calcium cations into the host cell. Translocation is probably initiated by interaction of CyaA monomer with the target membrane. The second activity is the formation of CyaA channel selective for cations, which probably causes colloid osmotic lysis of target cells. The channel forming activity is provided by RTX hemolysin domain which most probably forms oligomers, although it was found that CyaA as a monomer causes leakage of potassium cations from the host cell. It is also not clear whether the oligomerization of CyaA would occur in solution, or after interaction with the host membrane.

The aim of this study was to examine the flow of sodium ions on the membrane of murine macrophages J774A.1, which express integrin CD11b/CD18 on their surface. Fluorescence methods have been used to achieve this goal. Specifically, changes in concentration of sodium cations were monitored in the cytosol of J774A.1 cells labeled with probe SBFI/AM using emission ratiometric measurement or with probe Sodium Green by time resolved observation of lifetime changes.

Another goal of this work was to study CyaA oligomerization on different membrane systems, including the membranes of macrophages with CD11b/CD18 integrins. For this purpose we used the fluorescence method of measuring FRET in time resolution.