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Review

Channel formation by RTX-toxins of pathogenic bacteria: Basis of their biological activity☆

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ABSTRACT

The pore-forming cytolysins of the RTX-toxin (*R*epeats in *To*Xin) family are a relatively small fraction of a steadily increasing family of proteins that contain several functionally important glycine-rich and aspartate containing nonapeptide repeats. These cytolysins produced by a variety of Gram-negative bacteria form ion-permeable channels in erythrocytes and other eukaryotic cells. Hemolytic and cytolytic RTX-toxins represent pathogenicity factors of the toxin-producing bacteria and are very often important key factors in pathogenesis of the bacteria. Channel formation by RTX-toxins lead to the dissipation of ionic gradients and membrane potential across the cytoplasmic membrane of target cells, which results in cell death. Here we discuss channel formation and channel properties of some of the best known RTX-toxins, such as α-hemolysin (HlyA) of *Escherichia coli* and the uropathogenic EHEC strains, the adenylate cyclase toxin (ACT, CyaA) of *Bordetella pertussis* and the RTX-toxins (ApxI, ApxII and ApxIII) produced by different strains of *Actinobacillus pleuropneumoniae*. The channels formed by these RTX-toxins in lipid bilayers share some common properties such as cation selectivity and voltage-dependence. Furthermore the channels are transient and show frequent switching between different ion-conducting states. This article is part of a Special Issue entitled: Pore-Forming Toxins edited by Mauro Dalla Serra and Franco Gambale.

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1. Introduction

The RTX-toxins (*R*epeats in *To*Xin) represent a class of bacterial toxins with more than 1000 known species that exhibit various specific biological activities [1]. Typical for these bacterial toxins is a domain in the C-terminal half of the toxin proteins that contains a variable number of glycine-rich and aspartate-containing nonapeptide repeats of the consensus sequence G-G-X-G-(N/D)-D-X-(L/I/F)-X (where X can be any amino acid). The toxin proteins are called because of the repeats RTX (*R*epeats in *To*Xin) toxins [2,3]. The repeat domain binds calcium followed by the formation of ß-roll motifs shown in Fig. 1. This figure shows part of the ß-roll motif (amino acids 328–400) together with five Ca^{2+} binding sites of the alkaline protease of *Pseudomonas aeruginosa* [4]. The successive beta strands within the ß-roll motif wind in a right-handed spiral. Ca^{2+} ions are bound within the turns between two strands by a repeated GGXGXD sequence motif (where X is any amino acid), which means that each nonapeptide motif forms two half-sites for calcium ion binding between two turns (see Fig. 1). These structures are essential for biological activity via receptor-recognition on the mammalian target cells [4,5]. One subclass of RTX-toxins is of special interest here because of their high hemolytic and/or cytolytic activity, the pore-forming RTX-toxins. Alpha-hemolysin (HlyA) of *Escherichia coli* comprising 1024 amino acids is the prototype of this family of cytolysins from Gram-negative bacteria (see ref. [6] for a review). This RTX-toxin contains 13 repeats and efficiently lyses erythrocytes [2,6]. Pore formation by HlyA of *E. coli* is discussed here in detail because it is one of the best studied RTX-toxins. Besides this RTX-toxin pore formation by the Apx-toxins of *Actinobacillus pleuropneumoniae* will also be described together with the properties of adenylate cyclase toxin (Act, CyaA) of *Bordetella pertussis* that is another well studied RTX-toxin with 42 repeats because it has a two-fold function as pore former and as an enzyme that acts intracellularly to form cyclic AMP [7]. The pore-forming RTX-toxins are amphipathic proteins that have water-soluble precursors but can also form membrane-spanning structures. This means that the molecules undergo substantial structural changes. The mature RTX-toxins contain different domains responsible for export and biological activity (see Fig. 2B). Essential for hemolytic and cytotoxic activity of the RTX-toxins is the amphipathic domain near the N-terminal end that may form α-helical structures [8–13] but also ß-stranded domains seem to be possible [14]. However, the exact structure of the membrane pore formed by RTX-toxins is still a matter of debate and needs further elucidation.

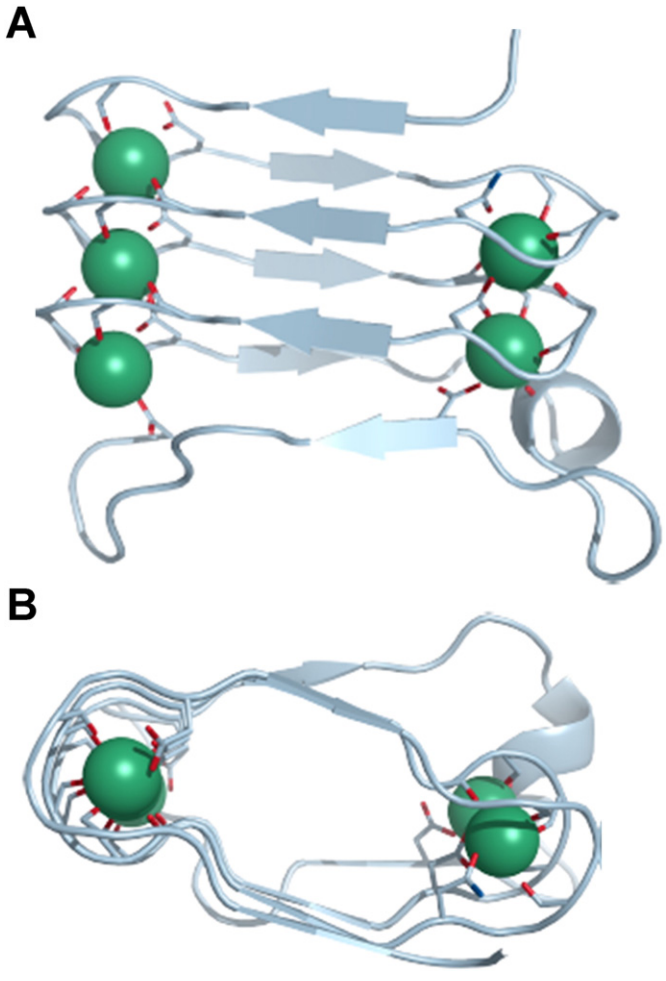

Fig. 1. 3D-structure of part of the calcium binding ß-roll motif of the alkaline protease of *Pseudomonas aeruginosa* modified after PDB ID: 1KAP.pdb [4]. Shown is the structure of the amino acids 328–400 of the protein given in light (lower strands) and dark blue (upper strands) for the ß-strands together with five binding places for calcium ions provided by two adjacent turns of the ß-roll motif (sequence GGXGXD where X is any amino acid). The 5 bound Ca^{2+} ions are given in dark green. Panel A shows the view perpendicular to the ß-strands. Panel B shows the view in parallel to the ß-strands.

Cytolytically or hemolytically active RTX-toxins have molecular masses between 100 and 200 kDa and are produced by Gram-negative bacteria [1–3,15,16]. The export across two membranes from the cytoplasm to the surface of the cells is mediated via type 1 secretion systems (T1SS) composed of different components that are encoded on the *rtx*-operon in transcriptional order (see Fig. 2A for the *rtx*-operon of HlyA of *E. coli*) (see ref. [17] for a recent review). The signal for export out of the bacterial cell is localized within the last 60 amino acids from the C-Terminus of the RTX-toxins [18,19]. This domain may also confer export by T1SS to chimera proteins not related to RTX-toxins but containing the export signal from HlyA at the C-terminal end [20]. The T1SS system is formed of an inner membrane ABC-transporter protein (HlyB in the case of *E. coli* HlyA) and an adaptor protein (HlyD) that links the inner membrane to the outer membrane export device. The outer membrane protein TolC also known as channel-tunnel is also part of the T1SS system but in the case of *E. coli* and many other RTX-toxins produced by Gram-negative bacteria it is not encoded by the *rtx*-operon itself but it interacts with it and with other export systems [3,17,21–23]. TolC represents a unique structure because its trimers contain two different parts: an outer membrane part that forms a 4 nm long β-stranded cylinder with 12 outer membrane spanning β-strands and a periplasmic α-helical cage with 12 α-helices that has a length of about 10 nm and spans presumably the whole periplasmic space [23]. In other cases, for example in the case of the *cya*-operon of ACT of *B. pertussis* and the *prt*-operon of the metalloprotease PrtA (a member the family of the RTX-metalloproteases [1]) of *Erwinia chrysanthemi* the *rtx*-operons contain also the genes for TolC homologous outer membrane proteins (*cyaE* and *prtF*) essential for export of the toxins out of the cells [7,24,25]. For some of the cytolytic and hemolytic RTX-toxins it is known that they are posttranslationally modified by cytoplasmic proteins encoded by the *rtx*-operon (see Fig. 2A; HlyC and related proteins) that act as acyltransferases together with an acyl carrier protein [26–28]. The acylation of at least one (K690) out of two lysines of HlyA near the repeat domain (K564 and K690) is essential for biological activity as cytolysin and/or hemolysin [29]. A similar essential role plays the RtxC proteins for the activation of other hemolytic and cytolytic RtxA proteins.

Extensive similarity has been demonstrated between the *rtx*-operons of *E. coli* and those of the Proteaceae [30,31], and also to those of the hemolysins and leukotoxins of *Actinobacillus* [22,32,33] and *Pasteurella hemolytica* [34] as well as the adenylate cyclase toxin (ACT, CyaA) of *B. pertussis* [7]. HlyA-proteins from different organisms exhibit immunological cross-reactions [35]. Several cell-based assays have shown that *E. coli* hemolysin is able to generate pores in mammalian

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