International Journal of Medical Microbiology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

Mini Review

Import and export of bacterial protein toxins

Volkmar Braun*, Stephanie Helbig, Silke I. Patzer, Avijit Pramanik, Christin Römer

Max Planck Institute for Developmental Biology, Department of Protein Evolution, Spemannstrasse 35, 72076 Tübingen, Germany

ARTICLE INFO

Keywords:
Colicin M
Pesticin
Hemolysin
Import
Export
Activity

ABSTRACT

The paper provides a short overview of three investigated bacterial protein toxins, colicin M (Cma) of *Escherichia coli*, pesticin (Pst) of *Yersinia pestis* and hemolysin (ShlAB) of *Serratia marcescens*. Cma and Pst are exceptional among colicins in that they kill bacteria by degrading the murein (peptidoglycan). Both are released into the medium and bind to specific receptor proteins in the outer membrane of sensitive *E. coli* cells. Subsequently they are translocated into the periplasm by an energy-consuming process using the proton motive force. For transmembrane translocation the colicins unfold and refold in the periplasm. In the case of Cma the FkpA peptidyl prolyl *cis-trans* isomerase/chaperone is required. ShlA is secreted and activated through ShlB in the outer membrane by a type Vb secretion mechanism.

Introduction

Bacteria secrete selected proteins by diverse, yet distinct mechanisms. For gram-negative bacteria at least seven protein secretion types and various subtypes are known. In addition, proteins exist which are secreted without a specific secretion mechanism. These are colicins which are released by nearly half of *Escherichia coli* natural isolates and kill competing sensitive cells. Despite co-synthesis of immunity proteins which confer resistance to the cognate colicins, a small proportion of cells in a population lyses when colicins are overproduced under stress conditions. In contrast to unspecific export highly specific and sophisticated colicin import systems exist in sensitive cells. According to their mode of action colicins enter the periplasm, the cytoplasmic membrane, or the cytoplasm of sensitive cells. For all colicins import starts with binding to specific receptor proteins exposed at the cell surface. Sensitivity of cells is largely determined by those receptors (Braun et al., 2002; Jakes and Cramer, 2012).

Here, two colicins from *E. coli* and *Y. pestis* and the hemolysin of *S. marcescens* were investigated by us and will be discussed with respect to their cellular export and import. Colicin M (Cma) and pesticin (Pst) were studied because only preliminary data existed and they differ from other colicins in that they primarily cause lysis of cells. All other colicins either degrade DNA or RNA in the cytoplasm or form pores in the cytoplasmic membrane resulting in collapse of the membrane potential and later cell lysis. Cma inhibits murein biosynthesis by cleavage of the ester bond in the murein precursor resulting in undecaprenol and 1-pyrophospho-MurNAc-(pentapeptide)-GlcNAc (Harkness and Braun, 1989; El Ghachi et al., 2006). These degradation products cannot be used anymore for murein biosynthesis. Pst cleaves the glycan chain of murein between C1 of MurNAc and C4 of GlcNAc (lysozyme activity; Vollmer et al., 1997). Plasmid-encoded Cma is made by *E. coli* and kills *E. coli* cells. Plasmid-encoded Pst is made by *Yersinia pestis* and kills Yersinia strains. It displays all characteristics of colicins and is, therefore, listed here as a colicin.

Hemolysin from *S. marcescens* has been included in our investigation (Braun et al., 1987) because it was at that time one of the few proteins that were secreted by Enterobacteriaceae and had not been investigated. The *S. marcescens* hemolysin structure and secretion mechanism turned out to be completely different from the well-studied *E. coli* hemolysin.

Domain structure of the *E. coli* colicin Cma

Cma consists of three domains which are characteristic of colicins. The central domain serves to bind Cma to the outer membrane receptor protein FhuA that concomitantly serves as a receptor for several phages and as a transporter for the iron chelator ferrichrome as well as for antibiotics derived from ferrichrome. The N-terminal domain is required for translocation across the outer membrane into the periplasm, and the C-terminal domain encodes the phosphatase activity (Fig. 1). Deletion of the hydrophobic α1 helix close to the N-terminus (Fig. 1; gray) abolishes binding of Cma to FhuA. However, phosphatase activity is retained as demonstrated in an assay that bypasses the FhuA function (Helbig and Braun, 2011). Mutations in α1 strongly reduce killing of cells. In contrast to wild-type Cma, these mutants do not prevent killing of

* Corresponding author.
E-mail address: volkmar.braun@tuebingen.mpg.de (V. Braun).

http://dx.doi.org/10.1016/j.ijmm.2014.12.006
1438-4221/

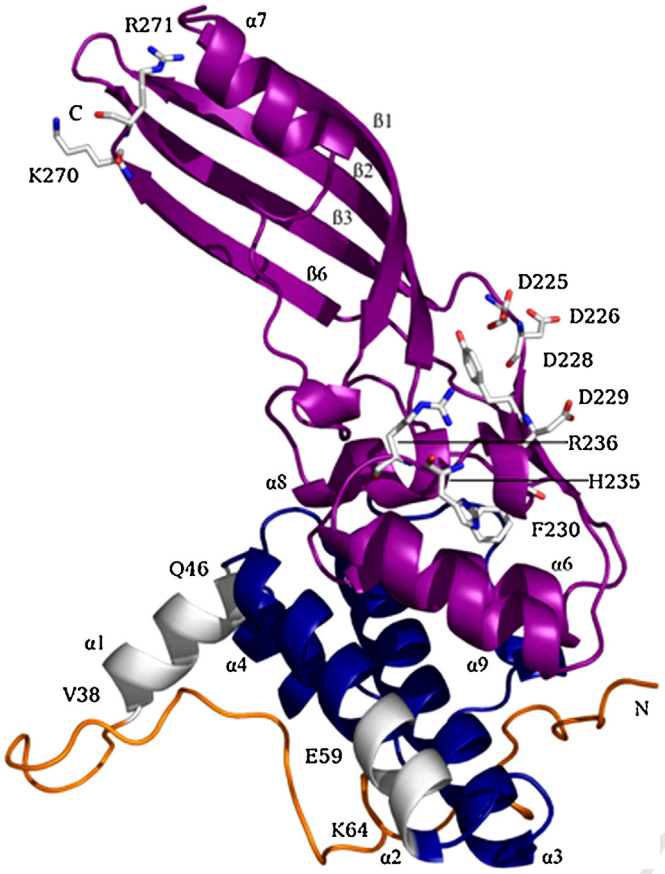

Fig. 1. Crystal structure (PDB 2XMX) of colicin M (Zeth et al., 2008) in which functionally important residues are indicated (Helbig and Braun, 2011). N-terminal translocation domain (yellow), central receptor binding domain (blue), C-terminal phosphatase domain (magenta),N-terminal end (N), C-terminal end (C) are indicated. Residues identified as important for Cma activity are shown as sticks. α1 indicates the region involved in receptor binding. α2 contains a strongly polar sequence, 59-EDYIKKH-65, but only the alanine replacement in E59 reduces Cma activity to 10%.

cells by the antibiotic albomycin, a ferrichrome derivative, which uses FhuA to enter cells. The mutant Cma proteins do not bind to FhuA which indicates that the α1 helix mediates binding of Cma to FhuA.

Sequence analyses of bacterial genomes reveal *cma* orthologs in strains of Pectobacteria, Pseudomonas and Burkholderia. The sequences are highly diverged in the receptor binding and translocation domains but similar in the phosphatase domain. The distinct receptor binding and translocation domains reflect individual import proteins in these bacteria. The conserved C-terminal region reflects the common killing mechanism. The three-domain structure also supports our previous conclusion that colicins in different bacteria evolved by horizontal gene transfer *via* plasmids and an exchange of gene fragments which encode functional domains (Braun et al., 2002; Roos et al., 1989). The phosphatase domain evolved from a single ancestor gene that was fused to distinct receptor binding and translocation genes.

Residues ^{226}DKYDFNASTHR^{236} are conserved, hydrophilic and exposed at the Cma surface (Fig. 1). Amino acid replacements in this region render Cma inactive. Particularly noteworthy are mutations D226E and D226N which completely inactivate Cma. This region is part of the active center of Cma, and D226 most likely is directly involved in hydrolysis (Pilsl et al., 1993; Helbig and Braun, 2011).

Import of Cma

Five chromosomally encoded genes are required for Cma killing of sensitive *E. coli* cells, *fhuA*, *tonB*, *exbB*, *exbD*, and *fkpA*. Mutations in any of these genes render cells resistant to high doses of Cma (dilution titers of 10^5). The outer membrane receptor FhuA is functionally coupled to the electrochemical gradient across the cytoplasmic membrane *via* the proteins TonB, ExbB and ExbD. These proteins are anchored in the cytoplasmic membrane and have distinct periplasmic domains. TonB physically interacts with FhuA, and ExbB and ExbD form a complex for which a stoichiometry of 6:1 was determined (Pramanik et al., 2011). It is hypothesized that FhuA changes its conformation in response to the membrane potential. Such conformational changes are suggested to result in release of high-affinity ligands (Cma, ferrichrome, albomycin, phages T1, T5, and Ω80) from FhuA into the periplasm. FhuA forms a β barrel composed of 16 β strands which is closed by an N-proximal globular segment termed plug or cork. The plug must move to allow ligand passage through FhuA. Currently it is unknown whether FhuA only acts as a primary adsorption site for Cma or also as the import route. Cma uptake is even more complex than the uptake of the other FhuA ligands as TonB must not only interact with FhuA but also with Cma (Pilsl et al., 1993). Cma contains a typical TonB box at the N-terminus. Point mutations in the TonB box abolish Cma uptake. Specific point mutations in TonB are capable to suppress point mutations in the Cma TonB box. This is taken as an indication for a direct interaction between the TonB box of Cma and the periplasmic domain of TonB. Outer membrane receptor proteins and all colicins which require TonB for activity contain an N-terminal consensus sequence, *i.e.* a TonB box. Suppressor analysis, cysteine cross-linking and crystal structures of TonB fragments bound to receptor proteins demonstrate a physical interaction with TonB (Braun, 2014).

Essential role of the periplasmic chaperone FkpA for Cma activity

FkpA is a peptidyl prolyl *cis-trans* isomerase with chaperone activity for which only very recently a control of outer membrane biogenesis under heat shock conditions has been assigned (Ge et al., 2014). We discovered that FkpA is essential for killing cells by imported Cma (Hullmann et al., 2008). Likewise, FkpA is essential for Cma secretion into the periplasm provided an artificial signal sequence has been attached to Cma (Helbig et al., 2011). For both, import and export Cma must unfold for crossing the outer and the cytoplasmic membrane. FkpA is indispensable for refolding and accelerates refolding of denatured Cma *in vitro*. Upon binding to FhuA Cma becomes sensitive to trypsin digestion. A mutation analysis of the fifteen proline residues in Cma identifies proline P176 as the most likely one which is *cis-trans* isomerized. Indeed, F175–P176 showed *in vitro* the fastest isomerization rate assayed with synthetic peptides derived from the Cma sequence. P176 is exposed on the surface of the Cma crystal structure which was determined for wild-type Cma and the inactive P176A mutant (Helbig et al., 2011). Of six inactive FkpA point mutants all are located in the isomerase domain. This data identify FkpA as essential for Cma *in vivo* activity and suggest that Cma is unfolded during import and refolded by FkpA involving *cis-trans* isomerization of the F175–P176 bond. Further, the data demonstrate an enzymatic activity for FkpA by which a proline bond is isomerized, and demonstrate for the first time an indispensable function of FkpA as a periplasmic chaperone in protein import.

Specific resistance to Cma by CbrA

Among Cma tolerant *E. coli* mutants (*tolM*), *fkpA* was identified (tolerance defined as Cma binding and, presumably, uptake, but no

Please cite this article in press as: Braun, V., et al., Import and export of bacterial protein toxins. Int. J. Med. Microbiol. (2014), http://dx.doi.org/10.1016/j.ijmm.2014.12.006