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Review

Pore-forming activity of clostridial binary toxins☆

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ABSTRACT

Clostridial binary toxins (*Clostridium perfringens* Iota toxin, *Clostridium difficile* transferase, *Clostridium spiroforme* toxin, *Clostridium botulinum* C2 toxin) as *Bacillus* binary toxins, including *Bacillus anthracis* toxins consist of two independent proteins, one being the binding component which mediates the internalization into cell of the intracellularly active component. Clostridial binary toxins induce actin cytoskeleton disorganization through mono-ADP-ribosylation of globular actin and are responsible for enteric diseases. Clostridial and *Bacillus* binary toxins share structurally and functionally related binding components which recognize specific cell receptors, oligomerize, form pores in endocytic vesicle membrane, and mediate the transport of the enzymatic component into the cytosol. Binding components retain the global structure of pore-forming toxins (PFTs) from the cholesterol-dependent cytotoxin family such as perfringolysin. However, their pore-forming activity notably that of clostridial binding components is more related to that of heptameric PFT family including aerolysin and *C. perfringens* epsilon toxin. This review focuses upon pore-forming activity of clostridial binary toxins compared to other related PFTs. This article is part of a Special Issue entitled: Pore-Forming Toxins edited by Mauro Dalla Serra and Franco Gambale.

Contents

1. Introduction

Clostridia are sporulating, anaerobic bacteria which are very widely spread in the environment (soil, sediment, water, dust, cadaver, litter, plant decomposition) including the digestive tract of man and animals.

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Among more than 200 *Clostridium* species, a few strains produce potent toxins and are responsible for severe diseases in humans and animals.

Bacterial protein toxins are defined as proteins secreted by certain pathogenic bacteria which are active at low concentration by inducing deleterious effects on target cells. The first step of the mode of action of bacterial protein toxins is the recognition of a specific receptor on target cells to which they bind with a high affinity. Based on their mode of action, two main classes of bacterial protein toxins can be distinguished, firstly those which act on cell membrane, for example through a pore-forming activity or enzymatic modification of membrane component(s), and secondly those which have the property to enter cells and modify intracellular target(s). Intracellularly active toxins contain at least three functional domains: receptor binding domain, translocation domain, and enzymatic domain. The receptor binding domain and translocation domain constitute the transport system that delivers the intracellularly active domain into the cytosol of a target cell. Numerous intracellularly active toxins are single chain proteins with at least three functional domains sequentially distributed on the toxin molecule forming distinct structural entities. In contrast, the binary toxins consist of two independent proteins encoded by two distinct genes, one coding for the binding component which contains the receptor binding domain and the translocation domain. Binding components are involved in the internalization into the cytosol of the corresponding enzymatic component coded by the second gene.

Binary toxins are produced by certain pathogenic species of the genus *Clostridium* (*Clostridium perfringens*, *Clostridium difficile*, *Clostridium spiroforme*, *Clostridium botulinum*), and by some *Bacillus* species (*Bacillus anthracis*, *Bacillus thuringiensis* and related species). *Clostridium* and *Bacillus* binary toxins exhibit some relatedness; particularly the binding components share related structure and mode of action. Notably, they use a common strategy to internalize the enzymatic component into the cytosol based on pore-forming activity of the binding components through the membrane of endocytotic vesicles.

2. *Clostridium* binary toxins

Four *Clostridium* species synthesize binary toxins as the only major toxin (*C. spiroforme*) or in combination with other toxins as in *C. perfringens*, *C. difficile*, and *C. botulinum*, and are mainly involved in intestinal diseases in man and animals.

2.1. C. perfringens Iota toxin

C. perfringens strains synthesize numerous toxins and are divided into five toxinotypes according to the main toxin(s) produced. *C. perfringens* type E secretes Iota toxin as major toxin which is constituted of a binding component (Ib) and enzymatic component (Ia). *C. perfringens* type E is responsible for enteritis and enterotoxemia in cattle and more rarely in other animal species. Overgrowth of *C. perfringens* in the intestinal tract of bovine under certain circumstances (microbiota equilibrium perturbation due to overfeeding, stress, rapid change in feeding, cold, etc.) results in production and secretion of Iota toxin in the intestinal content which then targets intestinal mucosa leading to cell necrosis and alteration of the epithelial permeability. In the late stage, Iota toxin can pass in the blood circulation leading to multiorgan failure [1,2]. The Iota toxin genes (*ia* and *ib*) are localized in large plasmids of various sizes in *C. perfringens* type E strains (reviewed in [3]).

2.2. C. spiroforme toxin

C. spiroforme strains only produce one type of toxin, *C. spiroforme* toxin (CST) which is composed of the binding component (CSTb) and enzymatic component (CSTa) [4,5]. *C. spiroforme* is an etiological agent of enteritis in rabbit which is characterized by watery diarrhea and death and is only rarely found in humans [6–8]. The two genes of CST (*cstA* and *cstB*) are chromosomally localized in *C. spiroforme* strains [4]

2.3. C. difficile transferase

Certain *C. difficile* strains produce a binary toxin called *C. difficile* transferase (CDT) in addition to toxin A and toxin B, which are part of the large clostridial glucosylating toxin (LCGT) family. *C. difficile* is recognized as the etiological agent of pseudomembranous colitis and about 30% of post-antibiotic diarrheas in humans, which are the main nosocomial infections of the digestive tract. *C. difficile* strains show a great genetic variability and are divided into multiple toxinotypes based on genetic variations on toxin A and toxin B genes. CDT was initially identified in a *C. difficile* strain responsible for a severe case of pseudomembranous colitis in 1997 in France [9,10]. It was shown that CDT is synthesized in addition to toxin A and toxin B by various toxinotypes and PCR-ribotypes including those considered as the most virulent and epidemic such as PCR-ribotypes 027 and 078 responsible for severe outbreaks of *C. difficile* infections [11]. From 2000 to 2003, the emergent PCR-ribotype 027 caused numerous and severe cases of *C. difficile* infections in North America and Europe. The as “epidemic” considered 027 ribotype produces toxin A, toxin B, as well as CDT. The 027 strains differ from the historical CDT-producing strain by a high fluoroquinolone resistance, by a 18 bp deletion, and a stop codon in the *tcdC* gene which encodes for an anti-sigma factor involved in the regulation of toxin A and toxin B gene expression [11]. A few *C. difficile* strains synthesize only CDT in the absence of toxin A and toxin B, but the role of CDT in *C. difficile* infection remains questionable. Indeed, strains only producing CDT have been isolated from patients with colitis supporting the view that CDT is involved in the pathogenesis. However, the incidence of *C. difficile* infections related to strains only producing CDT is low, and the symptoms are moderate. In addition, these strains yield no severe lesions of enteritis in experimental animals [11,12].

The two genes (*cdtA* and *cdtB*) respectively encoding each protein of the binary toxin CDT are localized on a specific region of the chromosome called CdtLoc (4.2 kbp) [9,13]. They are preceded by a regulatory gene termed *cdtR*, which is also included in CdtLoc [13], but the regulatory function of this gene is controversial [14].

2.4. C. botulinum C2 toxin

C. botulinum strains type C and D produce a binary toxin called C2 toxin which is clearly distinct from the neurotoxins C (or C1) and D. C2 toxin consists of a binding component (C2-II) and enzymatic component (C2-I) which disorganize the actin cytoskeleton through ADP-ribosylation of monomeric actin [15–18]. The C2 toxin component genes are localized on a large plasmid in *C. botulinum* C and D whereas the neurotoxin C and D genes are located on a phage. C2 toxin genes are expressed during the sporulation phase, in contrast to the neurotoxin genes which are expressed in the late exponential and early stationary phases [19,20]. The clostridial binary toxins (Iota, CDT, CST) are also synthesized during the exponential growth phase. Albeit structurally and functionally related to Iota toxin, C2 toxin shows a low identity at the amino acid sequence level and no cross immunogenicity with Iota toxin, CDT or CST. Thus C2 toxins, which exhibit sequence variations according to the *C. botulinum* strains are divided into three groups, and form a distinct family (C2 toxin family) from that of Iota toxin family (*C. perfringens* Iota toxin, CDT, CST) [21,22]. In addition, the binding components of Iota toxin family can mediate indistinctly the entry of the enzymatic component Ia, CDTa, or CSTa into cells but not C2-I, and reversely C2-II is only able to promote the cell translocation of C2-I [23,24].

C. botulinum C2 toxin induces intestinal hemorrhagic and necrotic lesions which are occasionally observed in animals, notably avian, that die