Molecular Genetic Analysis of Beta-Toxin of Clostridium perfringens Reveals Sequence Homology with Alpha-Toxin, Gamma-Toxin, and Leukocidin of Staphylococcus aureus

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Oligonucleotide probes designed on the basis of the N-terminal sequence of Clostridium perfringens beta-toxin were used to isolate the encoding gene (cpb). The nucleotide sequence of cpb was determined, and on the basis of DNA hybridization experiments it was shown that the gene is found only in type B and C strains of C. perfringens. The deduced amino acid sequence of the beta-toxin revealed homology with the alpha-toxin, gamma-toxin, and leukocidin of Staphylococcus aureus. The beta-toxin purified from C. perfringens appeared to exist in monomeric and multimeric forms. Recombinant beta-toxin, produced in Escherichia coli, appeared to be mainly in the multimeric form.

The etiology of diseases caused by Clostridium perfringens can be ascribed almost entirely to the potent exotoxins produced by the bacterium. Of the major toxins, alpha-toxin and epsilon-toxin are associated mainly with disease in humans and animals, respectively (17). In contrast, the beta-toxin is known to play a major role in the pathogenesis of necrotic enteritis in both hosts. In humans, the disease has been termed pigbel (12, 14, 15) and has been reported mainly in Africa, Southeast Asia, and New Guinea. It has been suggested that the disease occurs when the breakdown of beta-toxin, produced by C. perfringens in the gut, is prevented by the presence of trypsin-inhibitory compounds in the diet. Such trypsin inhibitors often originate from a diet rich in sweet potatoes. Necrotic enteritis attributed to beta-toxigenic C. perfringens has been reported in calves, lambs, and pigs (10, 33). Experimental infections when trypsin inhibitors are present in the diet have been reported in guinea pigs (14), but it is not clear whether the presence of dietary trypsin inhibitors is a prerequisite for disease in other animals.

Despite the significant association of beta-toxin with these diseases, little is known of the biophysical or molecular biological properties of the toxin; the toxin has been isolated in a pure form (21, 23) and shown to be lethal in mice (22) and guinea pigs (23). The mode of action of the toxin has not been elucidated, but the toxin has been shown to be dermonecrotic, to induce hemorrhagic necrosis in guinea pig intestinal loops (14), and to be cytotoxic for Chinese hamster ovary cells (13).

To study this toxin in more detail, we planned to isolate the encoding gene and determine its nucleotide sequence. This approach could facilitate the design of probes for the detection of the encoding gene and, in the longer term, the development of effective prophylactic or therapeutic reagents against beta-toxin-mediated diseases.

MATERIALS AND METHODS

Bacterial strains and cultivation. C. perfringens strains were cultured anaerobically at 37°C in a liquid medium containing Trypticase peptone (3%), yeast extract (0.5%), glucose (0.5%), cysteine hydrochloride (0.05%), and sodium thioglycolate (0.1%) that was adjusted to pH 7.5 by addition of Na₂HPO₄ (TPYG broth). For isolation of DNA, C. perfringens cells were cultured in 6-liter volumes of TPYG broth for 18 h and then separated from the culture fluid by filtration through a 0.22-μm-pore-size membrane. Escherichia coli JM101 and JM109 were stored and cultured as described by Sambrook et al. (25).

Preparation of DNA. Chromosomal DNA was isolated from the bacteria used in this study by the method of Marmur (16). DNA extracted from C. perfringens type B (NCTC 8533) was purified by centrifugation through a gradient of cesium chloride (25). Large- and small-scale plasmid isolations were performed as described by Sambrook et al. (25). Oligonucleotides were prepared with a Beckman 200A DNA synthesizer with the final trityl group left on. The oligonucleotides were purified with a cartridge purification system (Applied Biosystems).

N-terminal amino acid sequence of beta-toxin. Beta-toxin was purified from C. perfringens type C (CN 5386) as described previously (23). The N-terminal amino acid sequence of the toxin was determined independently at the U.S. Army Medical Research Institute for Infectious Diseases and the Faculty of Pharmaceutical Sciences, Tokushima Bunri University, with a gas-phase sequencer (470A; Applied Biosystems) equipped with an on-line phenylthiohydantoin analyzer (120A; Applied Biosystems).

Blotting of DNA and hybridization of gene probes. After electrophoresis, DNA digested with restriction endonucleases was transferred onto a nylon membrane (Hybond-N; Amersham). Undigested DNA was transferred onto the membrane with a slot blot apparatus. Oligonucleotide probes (10 pmol) were 5' end labelled (25). Unincorporated [γ-³²P]ATP was removed by passing the labelled oligonucleotide through a Nensorb 20 column [DuPont (UK) Ltd.] in
according with the manufacturer’s instructions. Isolated fragments of double-stranded linear DNA were labelled with \([\alpha-^32P]dCTP\) by using a random-primed labelling kit (Boehringer Mannheim UK Ltd.). The labelled probes were hybridized to membranes as described by Sambrook et al. (25). For oligonucleotide probing, the temperature of hybridization and washing was 42°C (ClosNH and ClosCOO), 34°C (Btox3), or 40°C (Btox2).

Cloning techniques. Two strategies were used to clone DNA fragments from C. perfringens type B (NCTC 8533). Chromosomal DNA digested with restriction endonuclease SspI was ligated to 0.1 µg of digested and dephosphorylated plasmid pUC18 and transformed into E. coli JM101 cells (9). Cells were transferred to a nylon membrane (Hybond-N) by colony blotting as described by Sambrook et al. (25), except that after replica plating, the colonies were incubated on agar containing chloramphenicol (100 µg/ml) to amplify the plasmid DNA.

Chromosomal DNA digested with HindIII or Sau3A was ligated to suitably digested and dephosphorylated M13mp18 (HindIII) or M13mp19 (Sau3A) (Pharmacia LKB Biotechnology) and transformed into E. coli XLI-blue (Stratagene), and membranes with E. coli JM105 TG1 cells were transferred onto plates of L agar plus ampicillin (25). The plaques were transferred onto a nylon membrane (Hybond-N) as described by Sambrook et al. (25).

After incubation of the membranes with radioactively labelled oligonucleotides as described above, colonies or plaques which hybridized with the probe were picked from the original plate and subcultured.

Nucleotide sequencing. Single-stranded DNA was prepared from M13 clones as described by Sambrook et al. (25). To generate single-stranded DNA from plasmid clones, the insert DNA fragments were amplified by using the polymerase chain reaction (PCR) with one of the oligonucleotide primers biotin labelled (Dynal A.S., Oslo, Norway). The biotin-labelled strand of DNA was isolated by using streptavidin-coated magnetic beads (Dynabeads; Dynal A.S.). Nucleotide sequencing was performed by the dideoxy-chain termination method with cloned T7 DNA polymerase (Sequenase Version 2.0; United States Biochemical Corp.) in accordance with the manufacturer’s instructions.

Alternatively, nucleotide sequencing of the positive strand from M13 cloned DNA fragments was performed by isolating the M13 replicative form (25), which then served as a template for use in a Taq polymerase cycle sequencing system (GIBCO-BRL).

Computer analysis of nucleotide and amino acid sequences. Nucleotide and deduced amino acid sequence data were analyzed with GENSTAR computer software. The amino acid sequence of Staphylococcus aureus alpha-toxin (8) was translated from the nucleotide sequence located in the EMBL sequence data base under accession no. X01645 and amended by changing the following residues: Gly-25 to Asn, Ala-74 to His, Iso-75 to Asn, and Glu-123 to Gln (13a). The amino acid sequences of beta-toxin and the individual S. aureus toxins were compared by using the PROSCAN and AALIGN programs (DNAStar). Multiple sequence alignments and comparisons were done with the Clustal algorithm within the MEGALIGN program (DNAStar). Hydrophathy (Hopp-Woods algorithm) and surface exposure (Emini algorithm) predictions were done with the appropriate DNAStar programs.

Expression of beta-toxin in E. coli. A DNA fragment which would encode the open reading frame of the beta-toxin and the proposed Shine-Dalgarno sequence was amplified from C. perfringens NCTC 8533 DNA by using the PCR. Oligonucleotides used in the PCR corresponded to nucleotides 316 to 338, with an additional 5’ region that encodes an EcoRI site (GTCGACGAATT), and the complementary sequence of nucleotides 1335 to 1355, with an additional 5’ region that encodes a PstI site (AAGCTTGGCTCGAC). A DNA fragment was obtained after 35 cycles of amplification (95°C, 15 s; 45°C, 15 s; 72°C, 30 s; Perkin Elmer 9600 GeneAmp PCR System 2400). The fragment was digested with EcoRI and PstI, ligated with suitably digested plasmid pUC19, and transformed into E. coli JM109 cells as described above. A colony containing the cloned beta-toxin gene (plasmid pBET7) was identified by using the PCR with the oligonucleotides described above. A culture of E. coli containing pBET7 was grown in L broth (37°C with shaking at 150 rpm) to an optical density at 600 nm of 0.5, at which time isopropyl-β-d-thiogalactopyranoside ( IPTG) was added to a final concentration of 1 mM and the culture was grown for a further 18 h. Periplasmic and cytoplasmic fractions of these cells were prepared as described previously (29).

SDS-PAGE and Western blotting (immunoblotting). Purified beta-toxin (100 µg/ml; 23) or extracts from E. coli containing pBET7 were mixed with sample buffer (4% [wt/vol] sodium dodecyl sulfate [SDS], 5% [vol/vol] β-mercaptoethanol), incubated (5 min, 22 or 100°C), and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) (0.1% SDS). In some experiments, the separated material was transferred onto a polyvinylidene difluoride membrane (Immobilon P; Millipore Corp.) and the membrane was blocked with a 5% (wt/vol) solution of dried skim milk in phosphate-buffered saline. The membrane was probed with a 1:1,000 dilution of a mouse anti-beta-toxin monoclonal antibody (F154/3-G7), and bound antibody was detected with a horse-radish peroxidase-labelled anti-mouse immunoglobulin G antibody (Sigma) by visualization of the bound antibody with a 1-chloro-4-naphthol substrate (25). In some experiments, horseradish peroxidase-labelled horse anti-C. perfringens type B serum (Wellcome Diagnostics) was used to probe the membrane directly, with visualization of the bound antibody as described above.

Nucleotide sequence accession number. The nucleotide sequence of the beta-toxin gene from C. perfringens has been submitted to GenBank and assigned accession no. L13198.

RESULTS

Design of oligonucleotide probes. Four oligonucleotides (ClosNH, ClosCOO, Btox2, and Btox3) were designed on the basis of the experimentally determined N-terminal amino acid sequence of beta-toxin. The probes were designed to be complementary to the coding (Btox2 and Btox3) or noncoding (ClosNH and ClosCOO) strand of the DNA (Fig. 1) and were selected from regions where possible codon degeneracy was minimal (26). To reduce the redundancy of two of the oligonucleotides further, the neutral base analog deoxyinosine (26) was incorporated into probe Btox3 at three positions and into Btox2 at two positions. The probes were also designed with reference to known codon usage preferences in C. perfringens (30) and, for ClosNH, ClosCOO, and Btox2, the ability of guanine and thymidine to pair with neutral bias. With these strategies, the probes were reduced to 128-fold (ClosNH), 64-fold (ClosCOO), 4-fold (Btox2), or 12-fold (Btox3) degeneracy.

Hybridization of oligonucleotide probes. DNA from C. perfringens type B (NCTC 8533) was digested with HindIII,
Sau3A, or SspI, and fragments which hybridized with βtox2 or βtox3 were identified by Southern blotting. The hybridization temperatures were calculated (26) to be 42°C (probes ClosNH and ClosCOO), 40°C (probe βtox2), and 34°C (probe βtox3). The probes all reacted with a 500-bp SspI fragment, a 700-bp Sau3A fragment, or an 850-bp HindIII fragment. In some experiments, probes ClosNH and ClosCOO reacted with a 1.4-kb Sau3A fragment. On the basis of these results, probes ClosNH, ClosCOO, and βtox2 were selected for isolation of the cloned DNA fragments because the higher hybridization temperature would minimize nonspecific probe binding.

Closing of the beta-toxin-encoding gene. DNA fragments generated after SspI digestion were cloned into plasmid pUC18, and approximately 5,000 ampicillin-resistant colonies were screened by hybridization with oligonucleotide βtox2. A single probe-reactive colony was isolated, the plasmid DNA was isolated (pBET400), and the identity of the DNA insert was confirmed by Southern blotting and reaction with probe βtox2. The nucleotide sequence of the probe-reactive fragment was determined; this revealed a sequence which could encode the N terminus of beta-toxin (Fig. 1A). Two differences between the experimentally determined and deduced amino acid sequences were observed, at positions 5 (threonine-lysine) and 13 (asparagine-lysine).

To isolate fragments of DNA that encode the remainder of the beta-toxin gene, fragments of DNA generated after Sau3A or HindIII digestion were cloned directly into bacteriophage M13. Approximately 10^7 plaques were screened from each library, of which 75 and 46%, respectively, appeared to contain insert DNA, as judged by inactivation of the lacZ gene. Twenty-five plaques which reacted with probes ClosNH and ClosCOO were identified, isolated, and plaque purified. Examination of the replicative-form DNA revealed that two of the HindIII clones (pBET2 and pBET4) contained a 0.85-kb fragment. A single Sau3A clone (pBET26), which contained a 1.4-kb DNA insert, was also selected for further characterization.

Nucleotide sequence determination. The nucleotide sequences of clones pBET400, pBET2, and pBET26 were determined by using a variety of sequencing strategies (Fig. 2). The nucleotide sequence of the pBET26 DNA insert was not determined at the 3' end. The nucleotide sequences of the three DNA fragments overlapped, and a total of 1,524 bp of the nucleotide sequence was obtained.

Features of the open reading frame. Examination of the nucleotide sequence of the cloned DNA fragments revealed the presence of a single open reading frame between nucleotides 333 and 1340 (Fig. 2). This open reading frame contained regions of DNA homologous with the oligonucleotides designed on the basis of the beta-toxin amino acid sequence. Comparison of the deduced amino acid sequence with the N-terminal amino acid sequence of beta-toxin indicated that amino acid Asn-28 corresponded to the start of the mature protein. The deduced amino acid sequence before this point showed features typical of a signal sequence (32); this region was characterized by a central hydrophobic domain (Fig. 3) and terminated with an alanine residue. Processing of the gene product, on export, would yield a protein with a calculated molecular weight of 34,861. The predicted isoelectric point of C. perfringens beta-toxin (5.5) was similar to previously reported values which had been experimentally determined (21, 35).

The region of DNA that encodes the beta-toxin (cpb) was A+T rich (71.5% A+T), which is close to the reported composition of C. perfringens DNA (73 to 76% A+T) (4)). A possible Shine-Dalgarno region was located 7 bp upstream of the ATG start codon of the cpb gene. This region could form a stable bond with the 3' end of the 16S rRNA from C. perfringens (ΔG = -20.2 kcal [1 cal = 4.184 J]). The region between the putative ribosome-binding site and the ATG start codon was composed entirely of thymidine residues. No potential promoter sequences were identified because of the high A+T content of the DNA upstream of cpb.

The nucleotide sequence downstream of cpb contained no regions predicted to form stable stem-loop structures which were followed by a poly(T) tract, suggesting that rho-independent termination of transcription did not occur.
Expression of cpb in E. coli. A DNA fragment which would precisely encode the beta-toxin open reading frame and the ribosome-binding site was generated from C. perfringens NCTC 8533 DNA by using a PCR with primers which bound upstream and downstream of the gene. The DNA fragment was cloned into plasmid pUC19 to generate plasmid pBET7. IPTG was added to cultures, and the cells were fractionated to determine the location of beta-toxin. The proteins were separated by SDS-PAGE and Western blotted with an anti-beta-toxin monoclonal antibody. A major reactive protein was detected in extracts from the periplasmic space of cells containing plasmid pBET7 but not in cells containing pUC19 alone (Fig. 4). Similar results were obtained when horse polyclonal anti-C. perfringens type B serum was used as the primary antibody. The apparent molecular mass of the main protein band was 118 kDa, and some immunoreactive bands with higher apparent molecular masses were also visible. A minor component with an apparent molecular mass of 34 kDa was also visible on Western blots. When beta-toxin purified from C. perfringens (23) was analyzed, two protein bands which reacted with the antibody, with sizes similar to those of the E. coli-expressed proteins, were detected. The 34-kDa component was the predominant immunoreactive form (Fig. 4). Incubation of the samples in sample buffer, without boiling, did not alter the profile of immunoreactive bands.

Homology of beta-toxin with other proteins. The deduced beta-toxin amino acid sequence was screened against the Swiss-Prot and PIR data bases with DNAStar computer software. We found significant homology with the deduced amino acid sequence of S. aureus alpha-toxin (13a) (28% similarity), the A, B, and C components of gamma-toxin (6) (22, 28, and 18% similarity, respectively), and the S and F components of leukocidin (19, 20) (17 and 28% similarity, respectively) and leukocidin S (27) (18 and 29% similarity, respectively) (Fig. 5). All of these proteins were similar in size (286 to 309 amino acids), and alignment of the deduced amino acid sequences revealed that 16 amino acids were identical and 95 were conservatively substituted. The homology between these proteins was weakest at the N termini (to residue 52 in Cpb).

Biophysical properties of beta-toxin and S. aureus alpha-toxin. In view of the amino acid sequence homology between C. perfringens beta-toxin and S. aureus alpha-toxin and the fact that both are single-component toxins, it was of interest to compare the predicted biophysical properties of these proteins. Both proteins were predicted to be composed
A monoclonal
Western were (lower trace) profiles of the unprocessed forms of C. perfringens beta-toxin (A) and S. aureus alpha-toxin (B). Positive values within the hydrophilicity plot indicate hydrophilicity, and negative values indicate hydrophobicity. The locations of predicted hydrophobic and surface-exposed regions are also shown boxed below the appropriate traces. The locations of areas of high amino acid sequence homology are indicated above the amino acid numbering. The hydrophobic region present only in beta-toxin is underlined.

mainly of a beta-sheet structure (48 and 49%, respectively), and circular dichroism studies have previously shown that alpha-toxin is composed largely (60%) of a beta-sheet structure (31). Comparison of the hydrophathy and surface exposure profiles revealed little overall similarity between these proteins (Fig. 3). To study the structural similarity between these proteins in more detail, the hydrophathy and surface exposure profiles within the highly homologous regions of these proteins were examined more closely and also compared with the profiles of the colocated regions in LukF and HlgB. The N terminus of the region between amino acids 80 to 102, region 146 to 148, and the C terminus of region 281 to 290 in Cpb were predicted to be surface exposed, and the colocated regions within Hla (Fig. 3), LukF, and HlgB (data not shown) were predicted to have similar properties. The region between amino acids 159 and 167 in Cpb and the colocated regions in Hla (Fig. 3), LukF, and HlgB (data not shown) were predicted to be hydrophobic and buried. Other regions of amino acid similarity between Cpb and the S. aureus toxins showed little structural similarity. The C termini of the S. aureus proteins were all predicted to be hydrophilic. The C terminus of Cpb was hydrophobic and could form a membrane-spanning region.

Probing of clostridial DNA for the beta-toxin gene. DNAs isolated from C. perfringens types A, B, C, and D were hybridized with radiolabelled plasmid pBET400 in a slot blot assay. The results (Fig. 6) indicated that the probe hybridized only with DNAs from C. perfringens types B and C. In other experiments, the probe failed to hybridize to DNA isolated from C. botulinum type B, E, or F; C. perfringens type E (CN5063); or C. novyi type A (NCTC 558) or D (NCTC 8350 or NCTC 9692) (data not shown).

DISCUSSION

Three degenerate probes designed on the basis of the N-terminal amino acid sequence of purified beta-toxin were used to isolate the beta-toxin gene (cpb) from C. perfringens NCTC 8533. The deduced N-terminal amino acid sequence of beta-toxin did not match the experimentally determined sequence precisely, indicating that the proteins from two different strains of C. perfringens were not identical. The differences observed (Thr-5 to Lys and Asp-13 to Lys) were not conservative substitutions. It is unlikely that these differences were due to errors in the N-terminal amino acid sequence, since this was determined independently in two laboratories. The importance of these residues for the biological activity of the toxin could not be elucidated, since we did not compare the specific toxicities of the proteins. DNA hybridization studies revealed that cpb was found only in strains of C. perfringens types B and C, and these types are associated with beta-toxin production. It has previously been reported that beta-toxin is plasmid encoded (7), and our method of isolating bacterial DNA would not have discriminated between chromosomal and plasmid species (11).

Beta-toxin is one of the main extracellular products of type B and C strains of C. perfringens. Some features of the upstream nucleotide sequence suggested that translation of cpb mRNA would be efficient: the possible Shine-Dalgarno sequence, identified upstream of the ATG start codon, would form a highly stable hybrid with the 3' end of the C. perfringens 16S rRNA. The intervening seven-nucleotide spacing would be optimal for gene expression (34), and the unusual poly(T) composition could limit secondary structure, thus aiding efficient translation (34). The failure to identify possible −10 and −35 promoter sequences upstream of the ribosome-binding site may be due to the high A+T content of this region. Alternatively, it is possible that the gene forms part of a larger operon, but the location of translational termination signals, in all three reading frames, upstream of cpb indicated that the 3' end of an open reading frame would be located at least 300 bp upstream of cpb.

Comparison of the start of the beta-toxin N-terminal amino acid sequence with the deduced amino acid sequence...
showed that the first 27 amino acids of the protein could encode a signal sequence directing export of the toxin across the cell membrane. This result is in accordance with the finding that beta-toxin appears in the extracellular fluid in cultures of *C. perfringens*. When allowance was made for removal of the signal sequence, *cpb* would encode a protein with a molecular weight of 34,861. Previous workers have found that the protein had apparent molecular masses of 20 to 42 kDa, as determined by gel filtration chromatography or SDS-PAGE (22, 23, 35). In this study, we found that beta-toxin purified from *C. perfringens* existed in two forms when analyzed by SDS-PAGE and Western blotting: a form with a molecular mass of 34 kDa appears to correspond to the monomeric gene product, and the high-molecular-mass form of the toxin (118 kDa) may be due to oligomerization of the toxin. The calculated molecular weight does not correspond to a divisible number of subunits, but this may be due to the anomalous migration of the oligomer in SDS-PAGE gels. Beta-toxin isolated from the periplasmic space of *E. coli* appeared to be mainly in the high-molecular-weight form, and this may be due to the difference in the physicochemical environments of the periplasmic space in *E. coli* and the culture extracellular fluid of *C. perfringens*.

Previous workers have reported amino acid sequence similarity between gamma-toxin and alpha-toxin (6) and gamma-toxin and leukocidin or leukocidin R (27). The sequence alignment presented here is the first that compares all members of this family of proteins, and this will aid future studies with these toxins. On the basis of comparisons of amino acid sequences of peptides derived from *S. aureus* Panton-Valentine leukocidin (27), it seems likely that the F and S components of this toxin also belong to this family of proteins. Several regions within the proteins showed high degrees of amino acid homology and were predicted to be surface exposed, suggesting that they are functionally essential. Identical amino acids located within these regions would be candidates for future investigations of structure-function studies with these toxins. Treatment of beta-toxin with thiol-modifying or oxidizing agents has been reported to abolish lethal activity (24), and as there is a single cysteine residue in the mature protein this is a candidate residue for future
structure-function studies. Several studies have been undertaken to modify essential amino acids in \textit{S. aureus} alpha-toxin chemically: arginine, lysine, and histidine residues appear to be necessary for biological activity (1), but it is not possible to draw conclusions concerning the location or significance of these residues in beta-toxin from the comparison of the deduced amino acid sequences. It has been proposed that \textit{S. aureus} alpha-toxin is composed of two domains separated by a glycine-rich hinge region (1). A glycine-rich region is present in all of the toxins (positions 133 to 138 in Cpb), suggesting that this region performs the same function in all of these proteins. The C-terminal region of alpha-toxin has been suggested to be involved in cell binding (2, 3), and although this region was predicted to be hydrophobic, it is of interest that the C terminus of beta-toxin was predicted to be hydrophobic and potentially membrane spanning.

Both beta-toxin and alpha-toxin are considered to be single-component toxins, whereas gamma-toxin and leukocidin require two separate components to elicit cytolytic activity. However, alpha-toxin can form membrane-active hexamers (18) and in this form it may be appropriate to consider it a multicomponent toxin in which all of the subunits are identical. It is not clear whether the monomeric and oligomeric forms of beta-toxin are toxic, and further studies are required to determine the biological properties and relationship of the different molecular forms.

The cytolytic activity of \textit{S. aureus} alpha-toxin, gamma-toxin, and leukocidin S against a variety of cell types is well characterized (1, 5, 27, 35), and alpha-toxin and gamma-toxin have been classified as pore-forming cytolsins (28). A crude preparation of beta-toxin isolated from the periplasmic space of \textit{E. coli} expressing \textit{cph} was not hemolytic for rabbit erythrocytes (data not shown), and Jolivet-Raynaud et al. (13) have reported that beta-toxin was cytotoxic but not cytolytic for Chinese hamster ovary cells and inactive against a variety of other cell types. However, some similarities between the toxic properties of alpha-toxin and beta-toxin are apparent from other reports. Fifty percent lethal doses of 1.87 and 0.68 μg, respectively, for mice have been reported (22, 36), and it has been suggested that both toxins elicit effects on the central nervous system (1, 13, 17, 35).

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