**Clostridium perfringens** Type A Enterotoxin: Characterization of the Amino-Terminal Region

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The amino-terminal region of the enterotoxin of *Clostridium perfringens* was investigated by automated sequence analysis. The primary structure results revealed that the enterotoxin is composed of a single polypeptide amino acid sequence. Computer comparison of a 20-residue sequence with a sequence library of reported proteins revealed no significant chemical similarities, indicating that the enterotoxin represents a unique polypeptide primary structure.

*Clostridium perfringens* produces at least 12 different toxins which are implicated as important in the pathogenesis of the organism (15). One of these toxins is a heat-labile enterotoxin which is produced by the organism during sporulation (2, 3). The enterotoxin induces fluid accumulation in ileal loops in animals (16) and diarrhea in humans affected by *C. perfringens* food poisoning (14, 18). Recent studies on the mechanism of action of this enterotoxin indicate that the enterotoxin structurally alters cell membranes and affects cell function (12, 13).

The enterotoxin from *C. perfringens* type A was previously reported to be a protein with a molecular weight of 35,000 (see review in reference 15). Enders and Duncan (4) demonstrated that the purified enterotoxin was free of fatty acids, nucleic acids, and carbohydrate (< 1.2%) but showed electrophoretic microheterogeneity and formed large-molecular-weight aggregates. These authors found that the experimentally determined molecular weight of aggregates in the presence of detergents corresponded to multiples of a subunit molecular weight of 17,500. Yotis and Catsimpoolas (19) obtained isoelectric focusing results which indicated that the enterotoxin was composed of two protein components; however, the results of Granum and Skjelkvale (5) indicated that the enterotoxin was a single polypeptide chain with a molecular weight of 34,000, as evidenced by sedimentation equilibrium analysis.

In view of differences in the reported subunit composition of the enterotoxin, we undertook amino acid sequence analysis of the amino-terminal region of the enterotoxin. In addition, we compared this region of primary structure with reported primary structures of other proteins to investigate any possible occurrence of chemical similarities.

Enterotoxin was prepared by the method of Granum and Whitaker (6), and its biological activity was determined as described by Stark and Duncan (17). The enterotoxin gave a single homogeneous peak of molecular weight 35,000 when subjected to gel filtration by high-performance liquid chromatography (HPLC) in 8 M urea (Fig. 1), similar to the procedure of Kato et al. (9). Amino acid compositional analysis was carried out as previously reported for cholera toxin (10). The compositional results were in good agreement with those published by Hauschild et al. (8) and Granum and Skjelkvale (5).

![Fig. 1. HPLC size exclusion chromatography of C. perfringens on a Bio-Rad TSK-250 column (7.5 mm by 30 cm) eluted at 1 ml/min with 0.05 M sodium phosphate buffer (pH 6.8) containing 8 M urea. Arrows indicate elution times of standard proteins: Kd = 0, thyroglobulin; 43,000, ovalbumin; 17,000, myoglobin; Kd = 1, phenylalanine.](http://iai.asm.org/Downloaded from http://iai.asm.org on September 29, 2017 by guest)
ed amino acid sequence analysis of two separate enterotoxin preparations was performed with an
updated Beckman 890B sequencer with the di-
lute Quadrol program (Beckman program
011576). Sequencer products converted to phen-
ylthiohydantoins were identified by HPLC and
amino acid analysis after back hydrolysis with
HCl (11).

Table 1 shows the results obtained from au-
tomated sequence analysis of native enterotoxin.
The occurrence of a single amino acid residue at
each position is consistent with a toxin com-
posed of one polypeptide chain. Furthermore,
the molar yield obtained by extrapolating the
repetitive yield to residue zero was 57 nmol.
This yield was nearly identical to the amount of
protein (60 nmol) applied to the sequencer based
on an enterotoxin molecular weight of 35,000.
However, sequencer yields can vary up to 60%,
depending on the nature of the protein. There-
fore, the sequencer results are in agreement with
a molecular weight value for the enterotoxin of
21,000 to 35,000. It should also be pointed out
that Granum et al. (7) found that dansylation of
the enterotoxin gave only a single spot that ran
near dansyl methionine sulfoxide when analyzed
by thin-layer chromatography.

Comparison of the partial amino acid se-
quence of C. perfringens enterotoxin with a
library of reported sequences showed no signifi-
cant similarities, indicating that the enterotoxin
represents a unique gene product. The library of
sequences used in the comparison was the pro-
tein data base of Dayhoff (1) updated to August
1981. However, comparisons of larger segments
of amino acid sequence, when available, will be
required to establish more definitively the possi-
ble occurrence of homology with other known
protein sequences. The sequence comparison
was carried out by computer analysis, utilizing
the SEARCH program of Dayhoff (1).

Computer sequence comparisons were conducted by M. O.
Dayhoff, National Biomedical Research, Georgetown Univer-
sity Medical Center, Washington, D.C. We thank Linda
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L. K. D. is a J. W. McLaughlin Fellow.

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**TABLE 1. Automated sequence analysis of C. perfringens enterotoxin**

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<th>Edman cycle</th>
<th>Residue</th>
<th>Amt of residue (nmol)</th>
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</table>

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a Results were obtained with a spinning cup se-
quencer (Beckman 890B updated). No corrections
were made for residue recoveries. Phenylthiohydan-
toin amino acid residues were established by HPLC
and amino acid analysis. Sequence analysis was re-
peated three times.

b Quantitation obtained by amino acid analysis after
back hydrolysis of phenylthiohydantoin amino acid
residues and by HPLC.

c Initial amount applied to sequencer as determined
by amino acid analysis, assuming a molecular weight
of 35,000.

d Residue charge established by HPLC.

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