A COMMON SUBUNIT STRUCTURE IN CLOSTRIDIUM BOTULINUM TYPE A, B AND E TOXINS

B. R. DasGupta and H. Sugiyama

Food Research Institute and Department of Bacteriology
University of Wisconsin, Madison, Wisconsin 53706

Received May 31, 1972

Summary: Tryptic activation of Clostridium botulinum type E progenitor toxin of 147,000 mol. wt. involves cleavage of the molecule into at least two polypeptides that are separable when the disulfide bond(s) linking them is reduced. The 50,000 and 102,000 mol. wt. of these chains compare with the 53,000 and 97,000 values of the disulfide-linked polypeptides of type A toxin of mol. wt. 145,000. Type E toxin resulting from trypsinization of its progenitor and naturally activated type A and B toxins have similar subunit structures.

C. botulinum produces serologically distinct neurotoxin types of similar, if not identical, pharmacological activity. The unique nature of this biological activity suggests the different toxin types could have a common basic configuration.

Type A, B, and E neurotoxins are synthesized as progenitor toxins whose specific toxicities increase following their activation by suitable proteases (1). If the toxin producing cultures are proteolytic (all type A and some type B strains), endogenous enzyme(s) of the culture activates the progenitor toxin to toxin (the form with maximum toxicity). If the cultures are non-proteolytic, as are all type E strains, activation of their progenitor toxin requires experimental treatment with an enzyme such as trypsin (EC.3.4.4.4) (1, 2).

This report shows a structural alteration of type E progenitor toxin during its activation with trypsin. It also shows that the resulting type E toxin and naturally activated type A toxin are composed of subunits which behave similarly in polyacrylamide gel electrophoresis (PAGE).

Type A toxin was obtained by DEAE-cellulose chromatography of crystalline toxin with 0.025 M NaH_2PO_4-Na_2HPO_4 buffer, pH 7.9 (3) and was of mol.

wt. 150,000 (4). Type E progenitor toxin was isolated from strain Alaska E43 and was an essentially pure preparation of protein of mol. wt. 150,000 (5). The type E progenitor toxin was incubated with or without trypsin (toxin:trypsin = 19:1, w/w) at 37° for 1 hr in 0.01 M Na_2HPO_4-NaH_2PO_4 buffer, pH 6.0. Trypsin was a preparation freed of chymotryptic activity (EC 3.4.4.5) by treatment with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (Worthington Biochemical Corp., Freehold, N. J.). The intraperitoneal mouse LD_{50}/ml of type E progenitor toxin increased from 7.6×10^2 to 6.6×10^4 during trypsinization ($_{ca}$ 90 fold activation).

Preparation of gels, electrophoresis, staining (12 hr), and destaining were according to Weber and Osborne (6). The 5% polyacrylamide gels were made with 0.1 M Na_2HPO_4-NaH_2PO_4 buffer, pH 7.05, containing 0.1% sodium dodecyl sulfate. All samples were readied for PAGE by adding to 0.5 ml protein solution 50 µl of 10% sodium dodecyl sulfate and 5 µl of β-mercaptoethanol or 5 µl of water. The reduced and unreduced mixtures were held in boiling water for 5 min, cooled and urea added (24 mg to 0.1 ml sample). Gels (6 x 94 mm) were first electrophoresed without samples for 1 hr at 8 mA/gel and the electrode buffer was replaced with fresh buffer. Samples of 50 µl were then applied and electrophoresis carried out for 6-7 hr at 8 mA/gel.

Molecular weight of proteins in the samples was established by comparing their electrophoretic mobilities with those of marker proteins myosin, β-galactosidase, rabbit muscle phosphorylase A, bovine serum albumin, and ovalbumin (6). When these markers were coelectrophoresed in a single gel, the plot of migration vs. logarithm mol. wt. was a straight line over mol. wt. range of 43,000 to 220,000. Internal marker of myosin or β-galactosidase was used in gels in which test samples were electrophoresed for mol. wt. determinations. To minimize reaction of a nonreduced test sample with the β-mercaptoethanol in the protein marker solution, marker was layered over the test sample just before turning on the current.

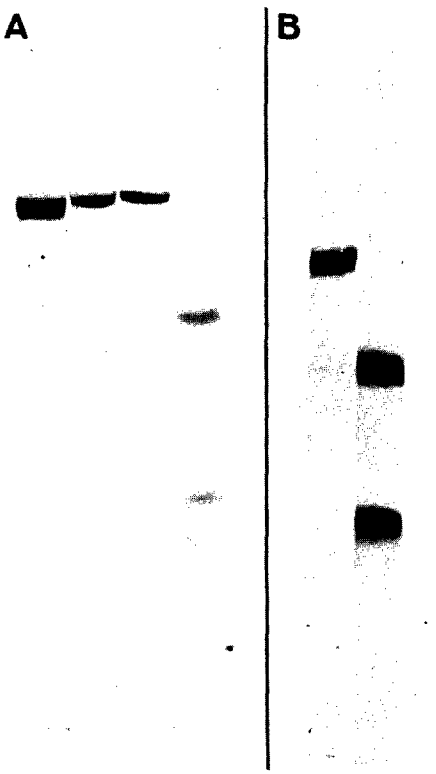

Fig. 1. Polyacrylamide gel electrophoresis of botulinum neurotoxins. Bands migrate from top to bottom (anode). A: type E samples. Gels from left to right: progenitor toxin without reduction, progenitor toxin after reduction, trypsinized progenitor toxin without reduction, and trypsinized progenitor toxin after reduction. Sample load 30-50 μg/gel. Trypsin band not visible. B: type A toxin. Left: 20 μg sample, not reduced; right: 40 μg, reduced.

Fig. 1A shows that both reduced and unreduced type E progenitor toxin migrated as one protein band in PAGE; these bands had similar mobilities. Type E progenitor toxin that was trypsinized but not reduced gave similar results. However, reduction of the trypsinized progenitor toxin resulted in the disappearance of the band produced by the unreduced sample and the appearance of two faster moving bands. Although not shown, electrophoresis of reduced or unreduced samples for shorter durations (2 or 4 hr) did not reveal any rapidly moving band which might migrate out of the gels during the routine 6-7 hr runs.

These results indicate that in the tryptic activation of type E progenitor toxin (mol. wt. 147,000 by PAGE) cleavage of at least one peptide bond occurs. This results in formation of two smaller polypeptides of mol. wt. 50,000 and 102,000. The bond cleaved is between two cystine residues forming an intrachain disulfide bridge; thus, the resulting polypeptide chains are held together until the disulfide is reduced. The possibility is not yet ruled out that more than one bond is cleaved; amino acids or small peptides that might be produced, if multiple bonds were cleaved, would not be detected by the PAGE technique.

The reported failure (2, 7) to detect breaking of a peptide bond during tryptic activation of type E progenitor toxin is not incompatible with the present results. The earlier work was based on trying to find a difference in the mol. wt. of untrypsinized and trypsinized samples, but reducing conditions were not used in the gel filtration and ultracentrifugation analyses.

Fig. 1B shows unreduced type A toxin migrated as one protein band in PAGE but upon its reduction the one band is replaced by two faster moving bands. Type A toxin (mol. wt. 145,000 by PAGE) is, therefore, composed of at least two polypeptides of mol. wt. 53,000 and 97,000 that are held together by disulfide bridge(s).

Type B toxin of mol. wt. 167,000 is composed of disulfide-linked polypeptides of mol. wt. 59,000 and 104,000 (8). Thus, a common structural feature is shared by the three well characterized botulinum toxins represented by naturally activated type A and B toxins and type E toxin obtained by trypsinization of its progenitor. Each has a light chain (mol. wt. 50,000 to 59,000) and a heavy chain (mol. wt. 97,000 to 104,000) that are held together by disulfide bond(s). The heavier chain is approximately twice the mol. wt. of the lighter.

As estimated by the PAGE method, the mol. wt. of type E progenitor toxin was 147,000; that of type A toxin was 145,000. These results agree well

with the 150,000 values found for both neurotoxins by gel filtration (2, 4, 5).

ACKNOWLEDGEMENT: We thank Dr. R. R. Rueckert and Miss Jane Hubertz of the Biophysics Laboratory for help in the initial electrophoresis tests. Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, by grant 5-R01-FD-00090 of the U. S. Public Health Service and contributions from various food companies.

REFERENCES:

1. Lamanna, C., and Sakaguchi, G. Bacteriol. Rev. 35, 242 (1971).
2. Kitamura, M., Sakaguchi, S., and Sakaguchi, G. J. Bacteriol. 98, 1173 (1969).
3. DasGupta, B. R., and Boroff, D. A. Biochim. Biophys. Acta, 147, 603 (1967).
4. DasGupta, B. R., and Boroff, D. A. J. Biol. Chem. 243, 1065 (1968).
5. Heimsch, R. C., and Sugiyama, H. Bacteriol. Proc. p. 68 (1971).
6. Weber, K., and Osborn, M. J. Biol. Chem. 244, 4406 (1969).
7. Sakaguchi, G. in M. Herzberg, ed, Proc. 1st U.S. - Japan Conf. on Toxic Microorganisms, U.S. Dept. of Interior, Washington, D. C. p. 341 (1970).
8. Beers, W. H., and Reich, E. J. Biol. Chem. 244, 4473 (1969).