Story of the BoNT/E Complex

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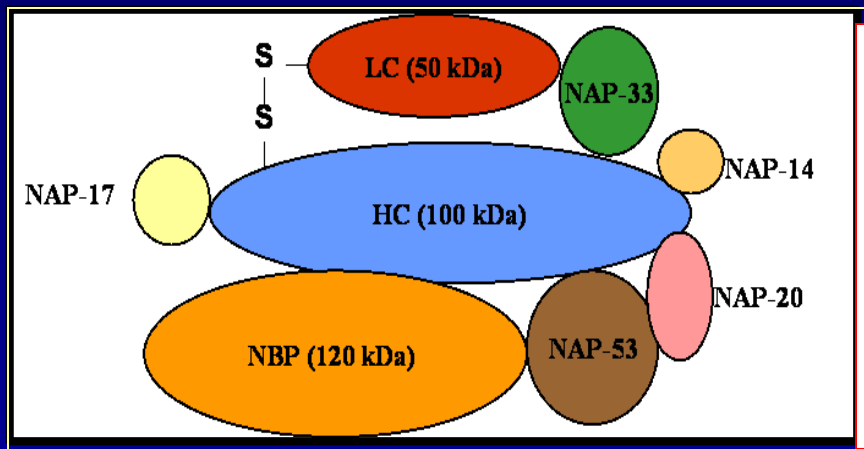
Botulinum neurotoxins in complex form

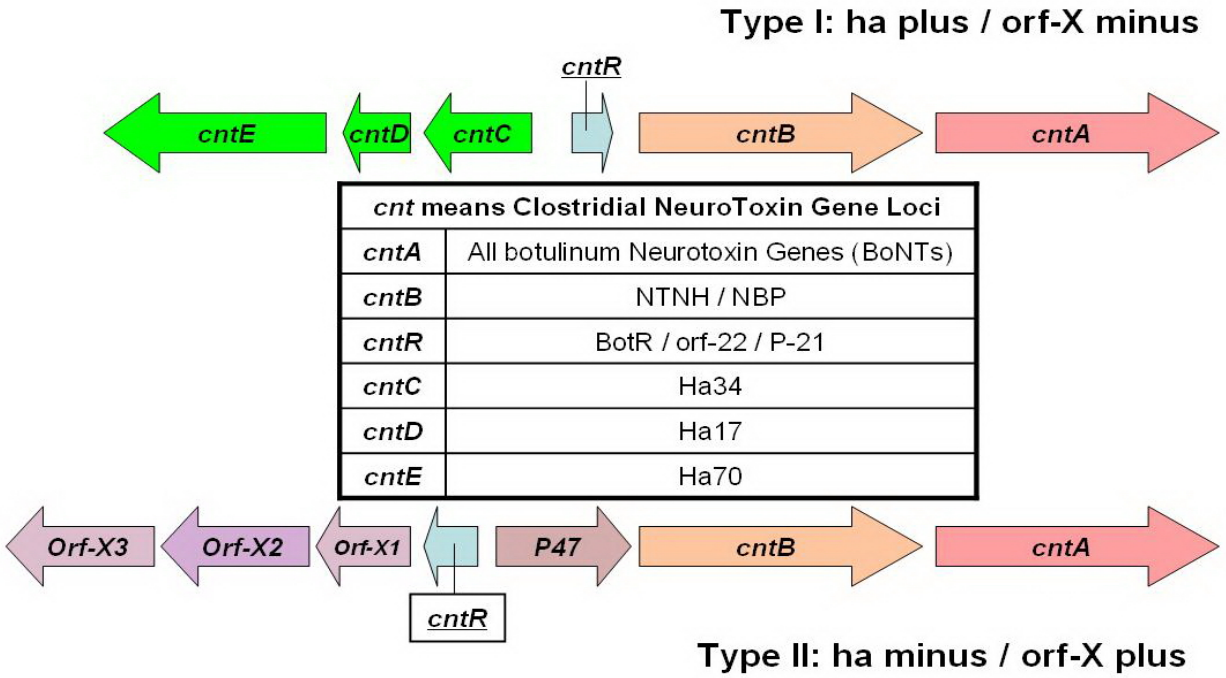
- Botulinum neurotoxins are secreted from the bacterium along with Neurotoxin Associated Proteins (NAPs)
- NAPs protect the toxin against proteases in GI tract
- NAPs play in critical role in intoxication



BoNT/A Complex
-BOTOX
-DYSPORT

BoNT/B Complex
-Neurobloc
-Myobloc

Schematic representation of BoNT/A complex



The *cnt* nomenclature system of botulinum neurotoxin gene cluster and its corresponding translation table for their earlier names.

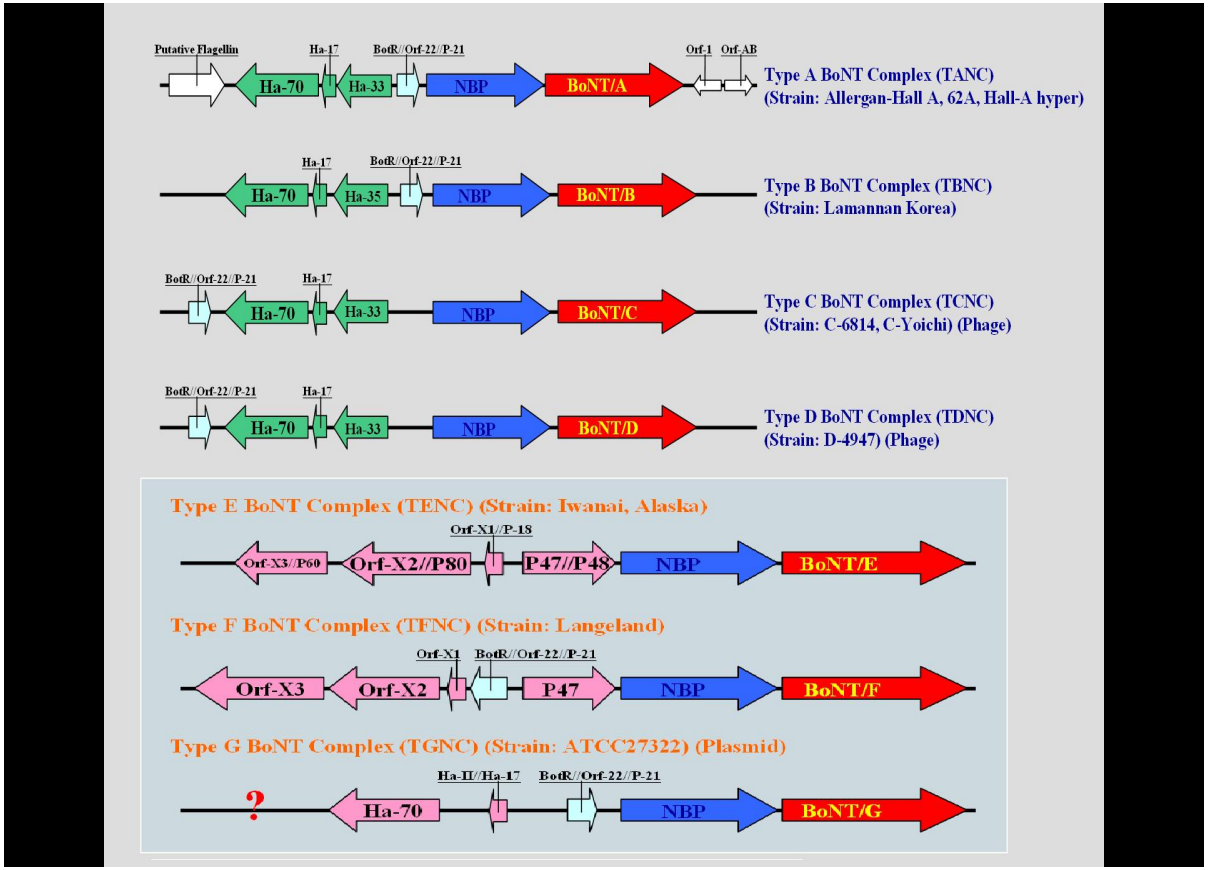


Figure The BoNT gene and its associated protein together form a polycistronic transcription unit. Red color represents the locus of BoNT gene and blue color represents the locus of NBP which co-transcribed in the same direction. The green color represents the hemagglutinin property and the loci of NAPs. The pink color means their hemagglutinin attribution hasn't been confirmed yet. The light blue color indicates the locus of transcription regulator for BoNT gene cluster. There are two types of BoNT gene cluster. Type I (HA+/Orf-) lacks Orf-X family (pink color, Orf-) protein but contains hemagglutinin (green color, HA+). Type II is the complementary (which means HA-/Orf+) form of type I.

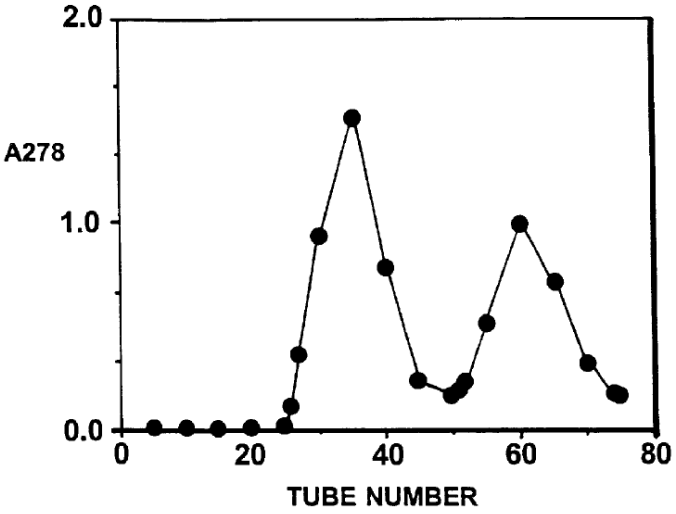


FIG. 1

Figure 1: Ion-exchange (DEAE-Sephadex A-50) chromatography on the type E C. botulinum cell extract. The column (4.8 x 53 cm) was equilibrated and eluted with 50 mM sodium citrate buffer, pH 5.5, at 20 °C. The flow rate was 40 ml/h, and the fraction size was 7 ml/tube.

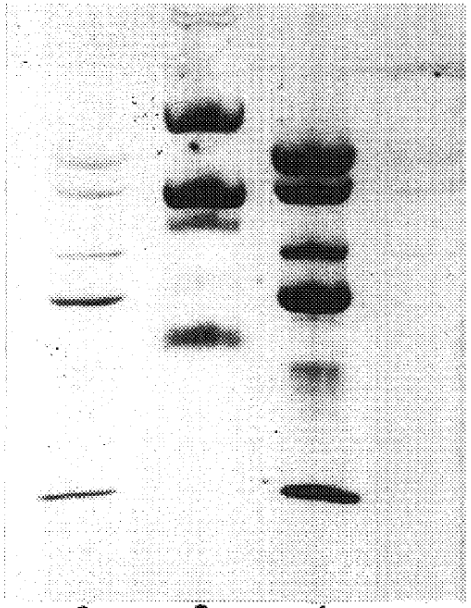


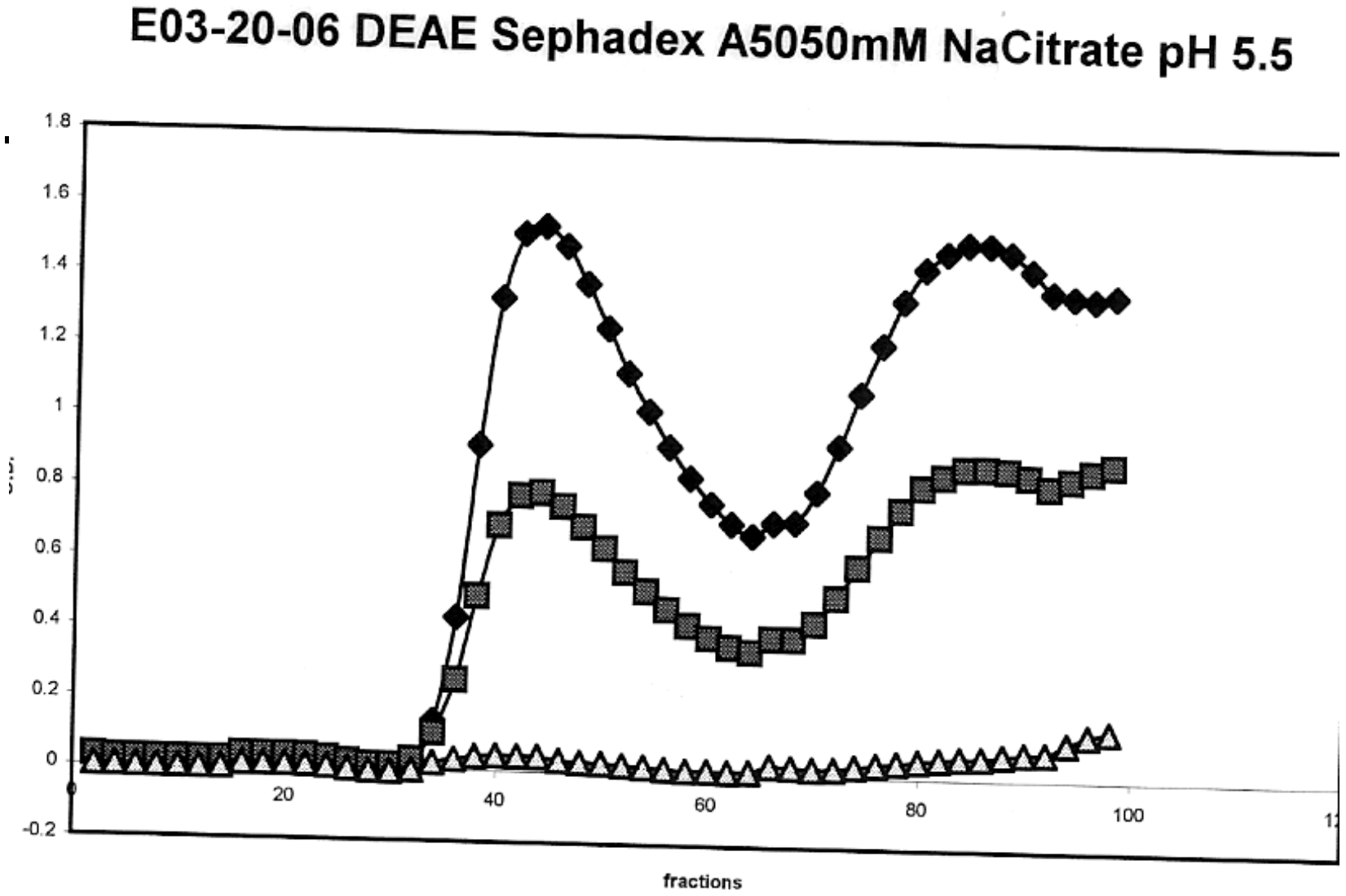
FIG. 2

FIG. 2 is a photograph of a polyacrylamide gel. Lane 1 was loaded with the material that eluted in the first peak of FIG. 1. Lane 2 was loaded with molecular weight standards. Lane 3 was loaded with material eluted from a G-200 column (see FIG. 5).

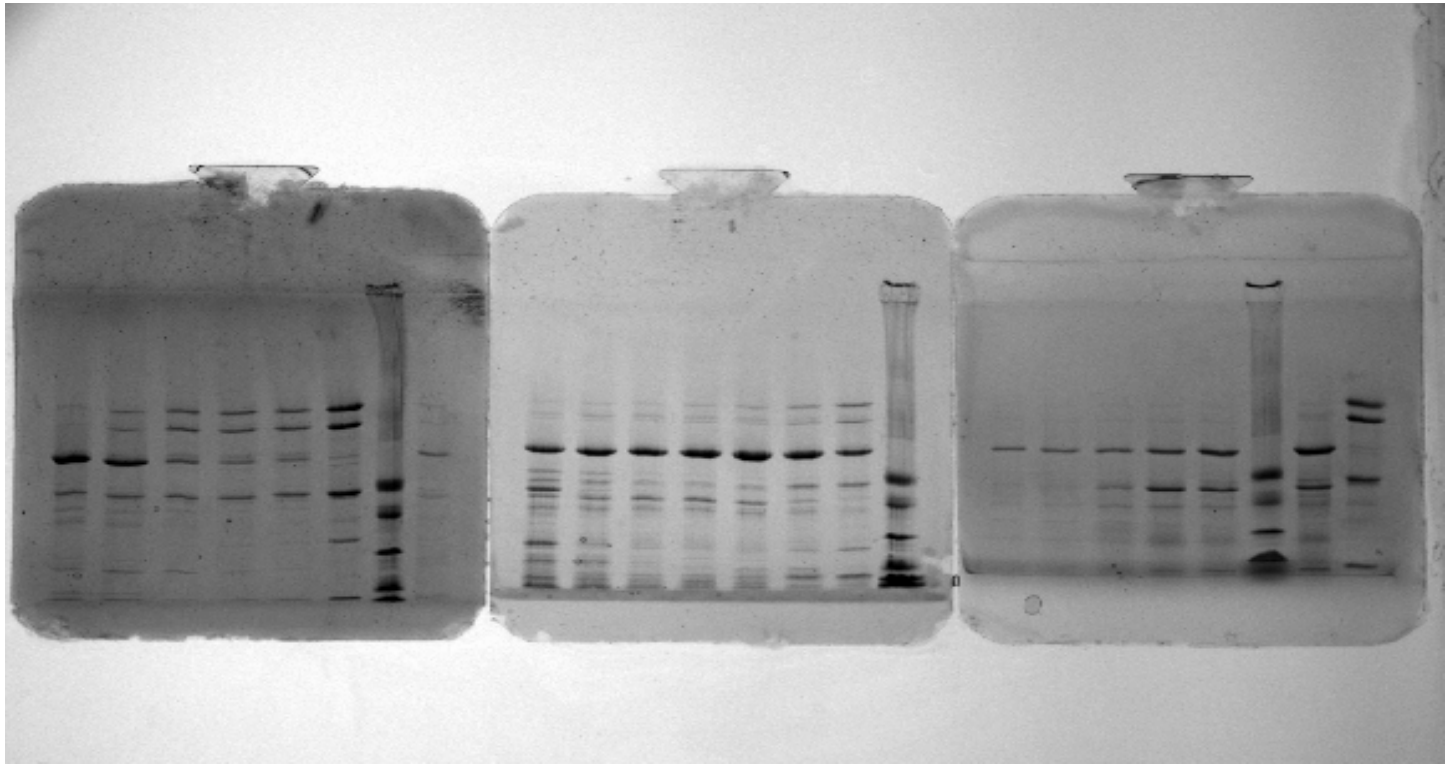
Isolation of “Crude Complex”

- DEAE Sephadex A50 is swelled and equilibrated with 0.05M Sodium Citrate pH 5.5
- Ammonium Sulfate precipitate form the preceding extracts are pelleted by centrifugation and dialyzed against the same buffer and subsequently loaded onto the aforementioned chromatography medium

Elution Profile for Sephadex A50



SDS – Page results for DEAE Sephadex A50



Gel 1 Load, BMW,Frac 44, 62, 64, 66, 68, 70
Gel 2 BMW, 72, 78, 84,90, 96, 102, 108
Gel 3 Frac 42, 114, BMW, 120, 126, 132, 133, 134
The following fractions were pooled
Fractions 34 – 66 approx. 54 mg
67 – 120 76 mg
121 – 134 13 mg

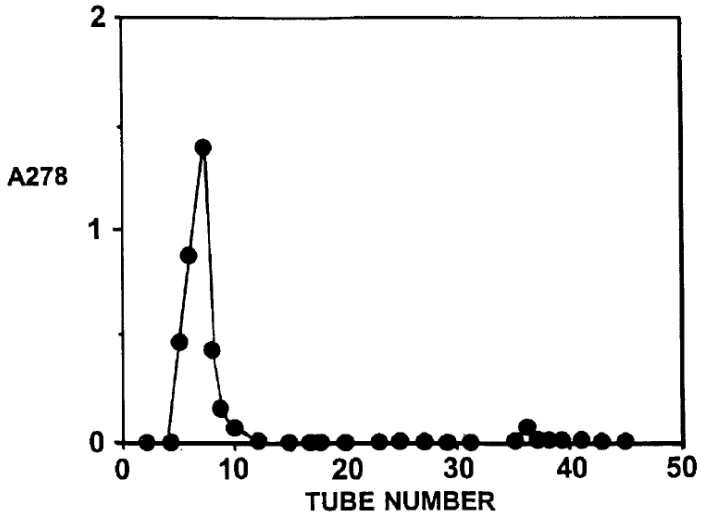


FIG. 3

FIG. 3 is an elution profile obtained by applying the type E botulinum neurotoxin complex to a Sephadex G-100 column.

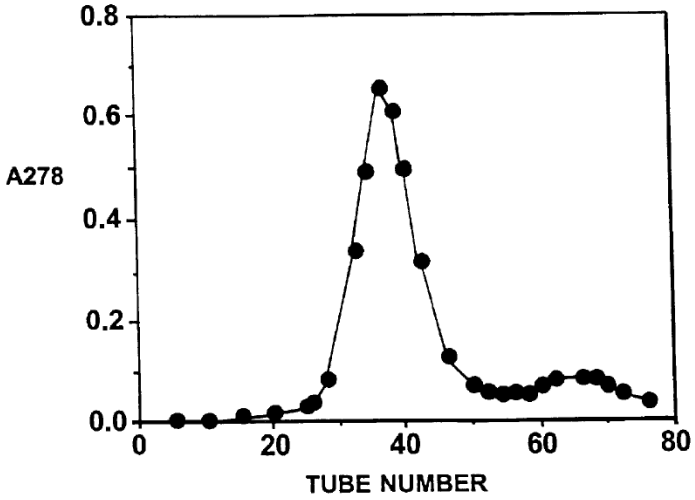


FIG. 4

FIG. 4 is an elution profile obtained by applying the type E botulinum neurotoxin complex eluted from a Sephadex G-100 column to a Sephadex G-200 column.

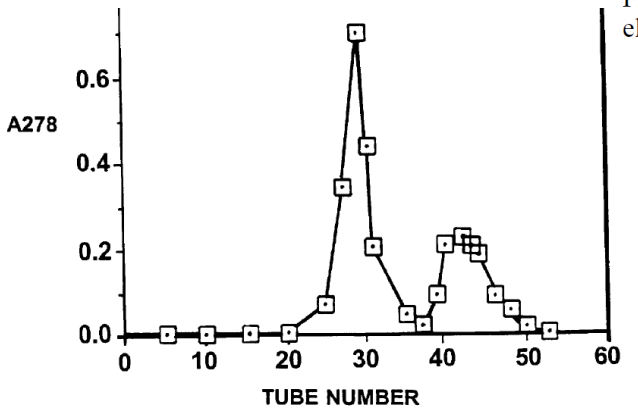


FIG. 5 is an elution profile of the complex formed between type E botulinum neurotoxin and the 80 kDa component of the associated protein complex.

FIG. 6 is a photograph of an SDS-polyacrylamide gel. The material in the first and second peaks of the elution profile shown in FIG. 5 was electrophoresed in lanes 1 and 2, respectively.

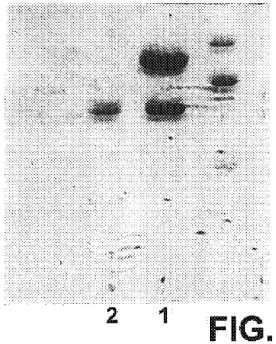
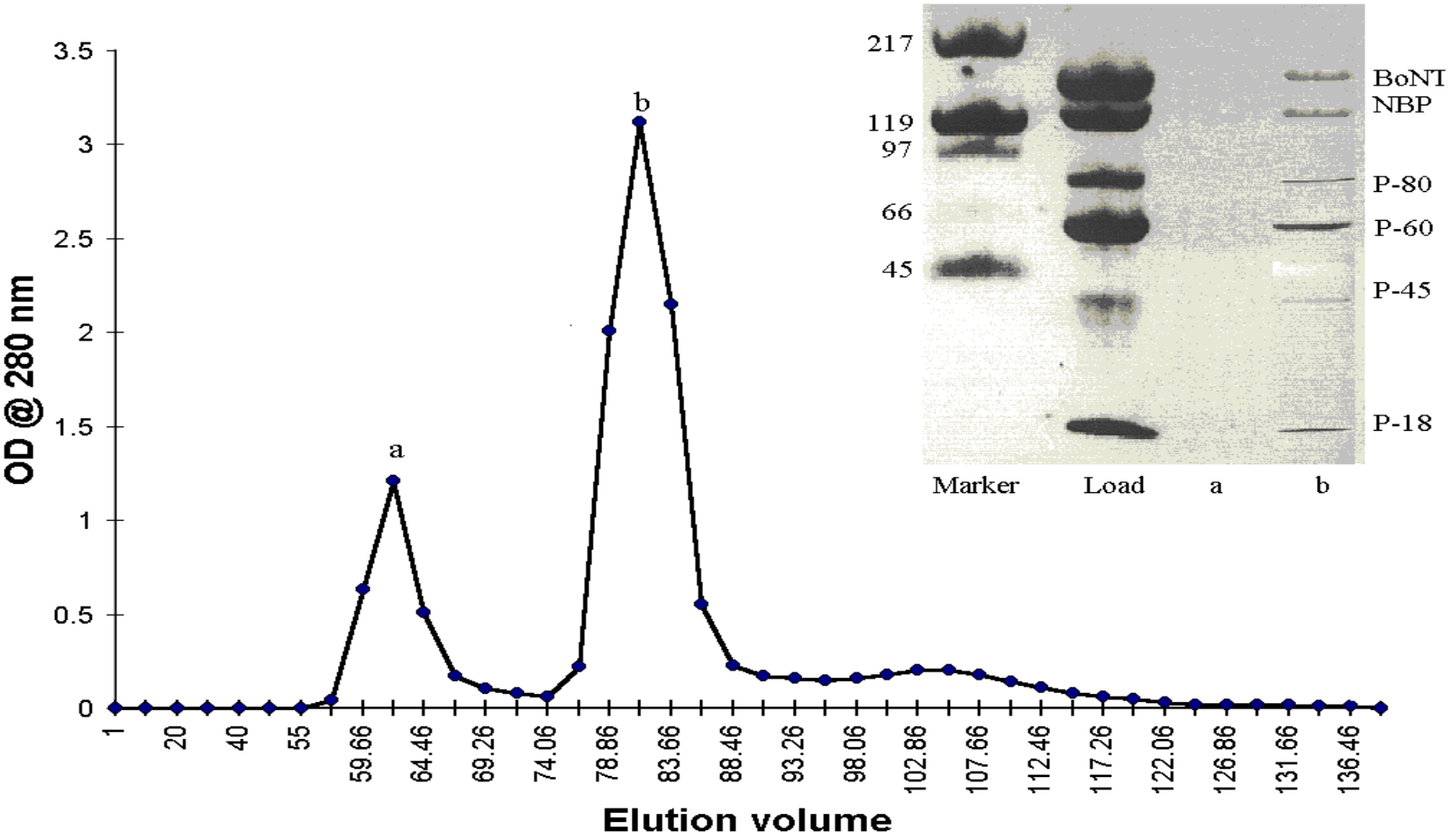


FIG. 6

♣ Determination of molecular size of novel type E neurotoxin complex



Molecular weight determination of type E complex by G-200 column.

Cloudy material was eluted in void volume (peak a).

Large BoNT/E complex, which is estimated as ~500kDa, is eluted in peak b.

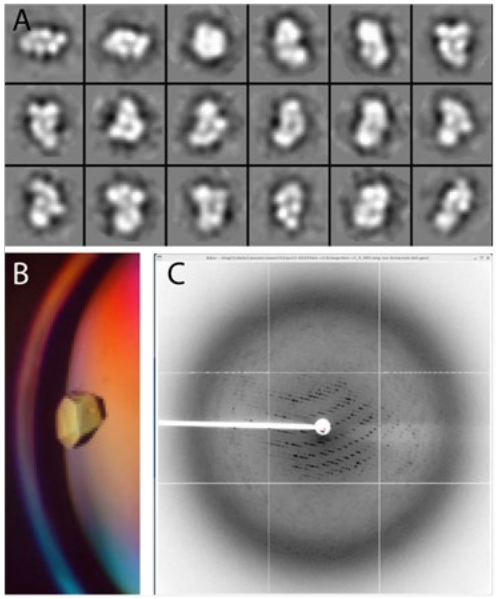


Fig. 6. (A) Selected class averaged EM images of purified PTC/E-L particles. (B) Preliminary PTC-E L-particle crystal. (C) Preliminary X-ray diffraction pattern

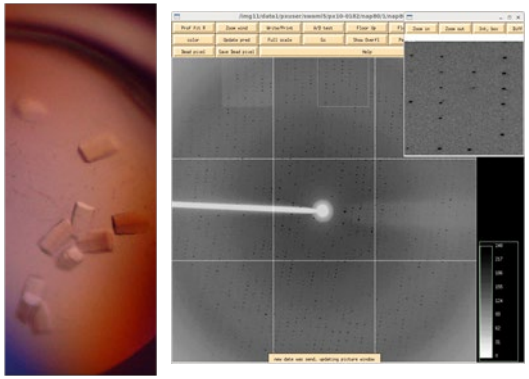
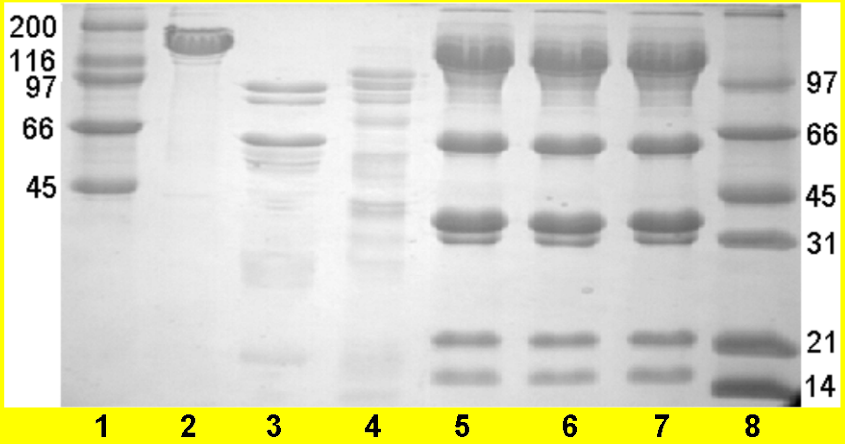


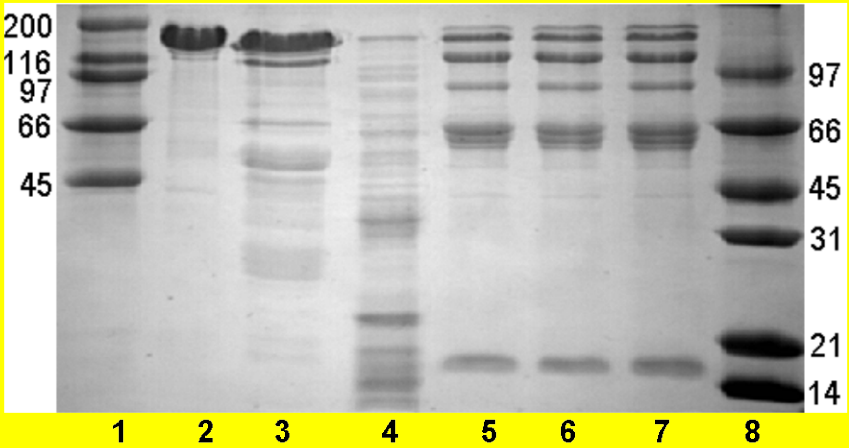
Fig. 8. Crystals and the x-ray diffraction pattern of NAP-80, the largest NAP in PTC-E(L). Crystals diffract to at least 3.1 Å. Inset shows

Inset shows zoomed view of the pattern near the edge.

Proteolysis of BoNT/A and E neurotoxin complex with Pepsin and α-chmyotrypsin.

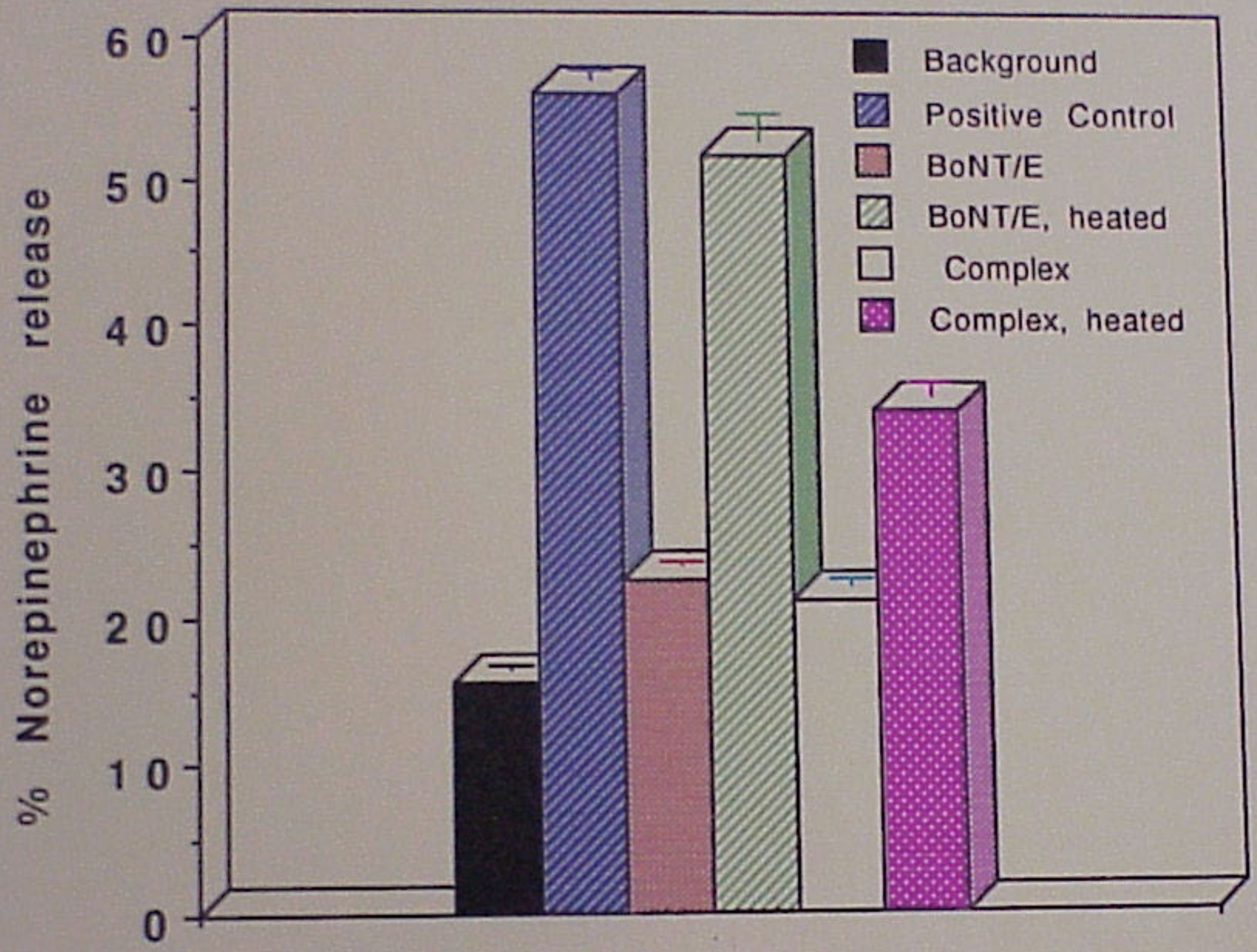


Complex A



Complex E

Lane 1: High molecular weight markers.
Lane 2: BoNT control.
Lane 3: BoNT treated with pepsin for 20 min.
Lane 4: BoNT treated with α-chmyotrypsin for 20 min.
Lane 5: BoNT complex control.
Lane 6: BoNT complex treated with pepsin for 1h.
Lane 7: BoNT complex treated with α-chmyotrypsin for 1 h.
Lane 8: Low molecular weight markers.


% Norepinephrine release
60
50
40
30
20
10
0
Background
Positive Control
BoNT/E
BoNT/E, heated
Complex
Complex, heated

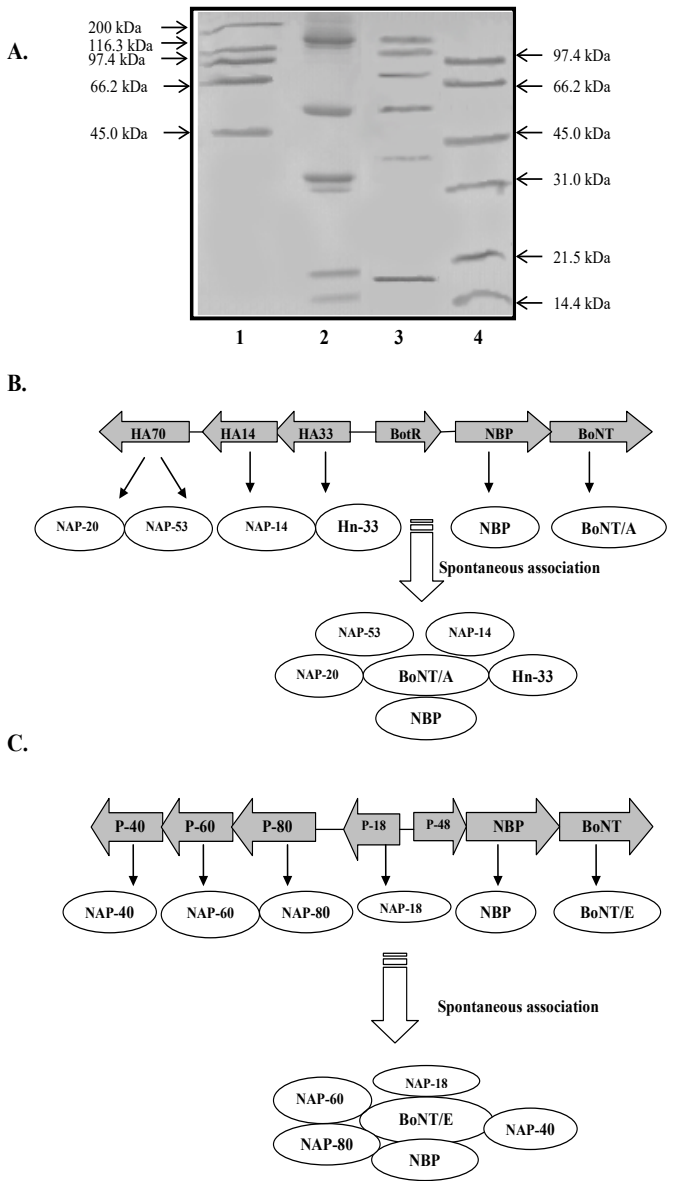


Figure: A, SDS-PAGE analysis of BoNT/AC and BoNT/EC under non-reducing conditions visualized by Coomassie blue staining: lane1: High molecular mass protein marker; lane 2: BoNT/AC; lane 3: BoNT/EC; lane 4: low molecular mass protein marker. **B,** Schematic diagram showing the genomic organization of *C. botulinum* type A (Fujinaga et al., 2004), and their expressed proteins in forming BoNT/AC. **C,** *Schematic* diagram showing the genomic organization of *C. botulinum* type E (Li et al., 1998) and their expressed proteins in forming BoNT/EC.

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