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Some Properties of Beta-Toxin Produced by *Clostridium perfringens* Type C

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Purified beta-toxin from *Clostridium perfringens* type C was found to be a single polypeptide chain protein with a molecular weight of approximately 30,000. The toxin was heat labile, with 75% of its activity being inactivated by incubation at 50°C for 5 min. Biological activity of the purified toxin was completely destroyed on exposure to trypsin for 30 min at 37°C. The 50% lethal dose for mice was 1.87 μg of purified toxin.

Beta-toxin is thought to be an important agent in necrotic enteritis of animals and humans caused by *Clostridium perfringens* type C. Several workers have reported conditions for beta-toxin production by this organism (3, 4, 7, 10, 11). Previously we reported the extensive purification of beta-toxin produced by *C. perfringens* type C (9).

To study the role of the toxin in necrotic enteritis, it is important to know the properties of the purified beta-toxin. Some of these properties are described in this report.

The strain of *C. perfringens* type C used was strain CN 5386, isolated from human cases of necrotic enteritis (the so-called "pig bel" disease) in New Guinea. Cultural conditions were as described previously (9). The procedure used for purifying beta-toxin involved ammonium sulfate fractionation, elution of the ammonium sulfate fraction from a Sephadex G-100 column, isoelectrofocusing of the beta-toxin fraction from the Sephadex G-100 column and immunoaffinity chromatography of the beta-toxin fraction pooled from an isoelectrofocusing column (9). Beta-toxin activity was determined in guinea pig skin as described previously (9). The purified beta-toxin was stored at −20°C for 3 months without detectable loss of the toxin activity.

The molecular weight of the purified toxin was estimated by gel filtration by the method of Andrew (1) which employs a Sephadex G-100 column (2.5 by 80 cm). Equilibration and elution of the column were done with 0.01 M phosphate buffer (pH 7.0). Bovine serum albumin, myoglobin, egg albumin (Miles Laboratories, Kankakee, Ill.), and deoxyribonuclease I (Sigma Chemical Co., St. Louis, Mo.) were used as standards. The standard proteins were assayed by their absorbance at 280 nm. The void volume was determined with blue dextran 2000 (Sigma Chemical Co., St. Louis, Mo.). The molecular weight of the toxin was estimated as approximately 30,000, as shown in Fig. 1. When the ammonium sulfate fraction of beta-toxin was eluted from a Sephadex G-100 column, alpha- and beta-toxins were eluted simultaneously as described previously (9). This compares to the molecular weight values of alpha-toxin, which Bernheimer and Grushoff (2) and other workers (6, 12) estimated with gel filtration as ranging between 26,000 and 31,000.

Thermostability of the purified toxin was also studied (Fig. 2). When the toxin was incubated in 0.01 M phosphate buffer (pH 7.0) at 50°C, a 75% decrease in toxin activity occurred within 5 min. After 60 min of incubation, more than 95% of the initial activity was lost. The data show that beta-toxin is heat labile.

Beta-toxin in culture supernatant fluids has been thought to be destroyed by trypsin during toxigenic typing of *C. perfringens*. As shown in Fig. 3, when the purified toxin was treated with trypsin at 37°C in 0.01 M phosphate buffer (pH 7.0), toxin activity was almost completely lost within 30 min. Thus, purified toxin is susceptible to trypsin.

Sodium dodecyl sulfate disc gel electrophoresis of the purified toxin was carried out by the method of Laemmli (5). The purified toxin gave a single band in this system. The data show that the toxin probably consists of a single polypeptide chain, because if multiple subunits existed, they would be disassociated by the sodium dodecyl sulfate treatment, resulting in multiple bands on electrophoresis.

Twofold dilutions of the toxin in 0.01 M phos-

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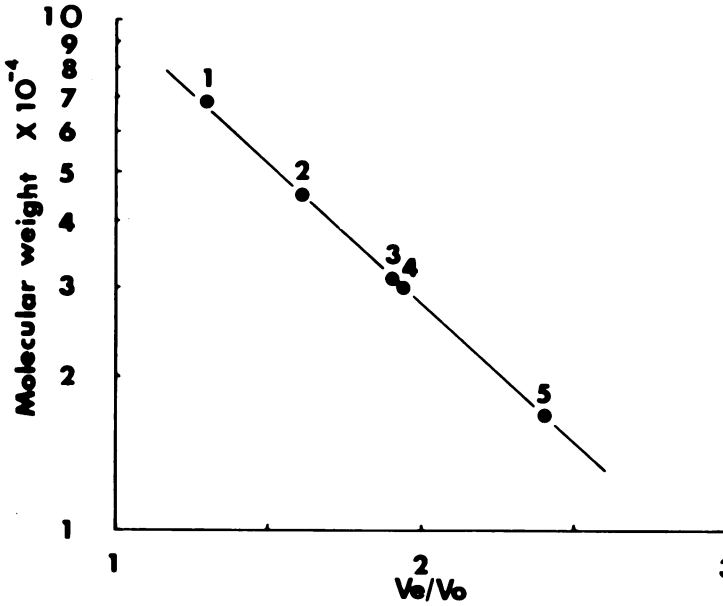

FIG. 1. *Determination of the molecular weight of purified beta-toxin by Sephadex G-100 gel filtration. Vo is the void volume of the column, and Ve is elution volume of each substance. 1, Bovine serum albumin; 2, egg albumin; 3, deoxyribonuclease I; 4, purified beta-toxin; 5, myoglobin.*

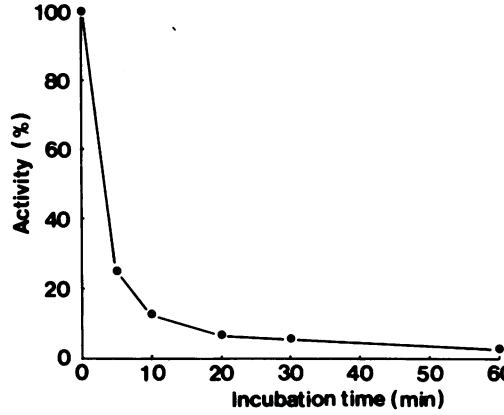

FIG. 2. *Thermostability of purified beta-toxin. Purified beta-toxin (150 μg/ml) was incubated in 0.01 M phosphate buffer (pH 7.0) at 50°C.*

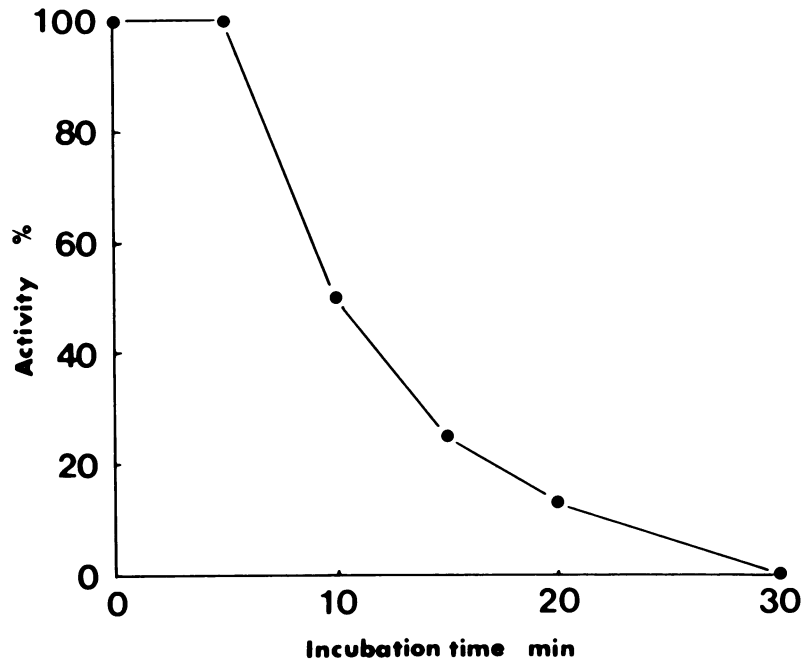

FIG. 3. *Effect of trypsin on the activity of purified beta-toxin. A 150-μg amount of purified beta-toxin was incubated with 300 μg of trypsin in 0.01 M phosphate buffer (pH 7.0) at 37°C.*

phate buffer (pH 7.0) were injected intraperitoneally into 20-g white male mice. A total of 0.5 ml of each dilution was injected into each of six mice, and the number of deaths was recorded after 48 h. The lethal dose for 50% of the animals was calculated by the method of Reed and Muench (8). The 50% lethal dose for mice was 1.87 μg of protein.

The data presented here are the first published report of some of the characteristics of purified beta-toxin.

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