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Short Communication

Isoelectric Focusing of *Clostridium botulinum* Type A Toxin

F. GALDIERO, M. A. TUFANO and T. CERCIELLO

Institute of Microbiology, The University of Naples, Naples (Italy)

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Isoelectric focusing for the analytical separation of proteins from *C. botulinum* culture, precipitated with ammonium sulphate solution, has demonstrated the presence of four fractions with different isoelectric points, two of which are highly toxic.

A strain of *Clostridium botulinum* type A isolated and identified by the laboratory from a fatal case of botulinism was used.

The organisms were grown for toxin production by the method described by Gerwing *et al.* (1961). Saturated ammonium sulphate solution was added to toxic filtrates to a final concentration of 50% (v/v). The precipitate was allowed to form overnight at 4° C and was then collected by centrifugation at 5000 × g at 4° C. The sedimented material was resuspended in H_2O in approximately 2% of the original volume. The misty suspension was added to a less dense ampholytes solution where it is cleared. The normal method described by Vesterberg *et al.* (1967) was used to separate in column of 110 ml capacity for isoelectric focusing (LKB Produkter, Stockholm-Bromma, Sweden). The carrier ampholytes were selected to give a pH gradient between pH 2 and 12. After focusing for 36 h with a final potential of about 400 V, the pH of the fractions were measured with a Beckman Expandomatic pH-meter. The amounts of protein in the fractions were estimated by measurements of optical density at 280 mμ.

The fractions were then neutralized and tested for toxicity. White mice weighing approximately 20 g were injected intraperitoneally with 0.5, 0.3, 0.2 and 0.1 ml amounts of the given toxic fraction diluted decimally in physiological saline. Groups of five mice were used per dose, the MLD was calculated as the highest dilution by which all mice were killed within 72 h.

With isoelectric focusing it is possible to separate the different fraction shown in Fig. 1. 4 fractions are obtained with pI spectra of

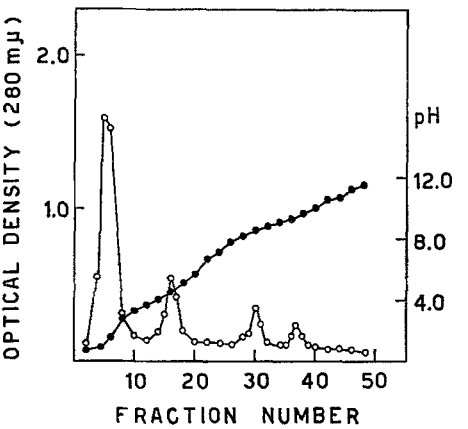

Fig. 1. Optical density at 280 mμ, o——o and pH at 4° C, •——• of the fractions taken from a 110 ml column after isoelectric focusing

between 1.2 and 1.8, 4.3 and 4.7, 8.3 and 8.7, 9.4 and 9.8. Fractions 1 and 4 are not toxic with mice, whereas fraction 2 and 3 with pI spectra 4.3 to 4.7 and 8.3—8.7 are highly toxic.

The broth culture precipitate has a specific activity of 1×10^5 mouse MLD per mg of protein, fraction 2 one of 2.5×10^6 and 3 of 5.7×10^6 mouse MLD per mg of protein. Electrofocusing separation is therefore a rapid method to obtain a high specific activity product.

The pI spectrum, especially from the acid point range, is somewhat different from that reported in the literature (pI between 6.5 and 8). Evidently with electrofocusing gives a separation of the toxic protein fraction composing the toxine in which some dissociable groups are buried. We have not enquired whether the two toxic fractions isolated can be identified with α and β obtained from crystalline toxine fractions.

References

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Dr. Francesco Galdiero
Istituto di Microbiologia
dell' Università di Napoli
Sant'Andrea delle Dame, 2
80138 Napoli, Italia