CLOSTRIDIUM BOTULINUM TYPE D TOXIN: PURIFICATION, CHARACTERIZATION AND COMPARISON WITH TYPE C_1 TOXIN IN MOLECULAR PROPERTIES

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Toxin of *Clostridium botulinum* type D strain 1873 (D_{1873} toxin) was purified from its culture supernatant in an overall yield of 32% ammonium sulfate precipitation and column chromatographies of Sephadex G-75, diethylaminoethyl cellulose, quaternary aminoethyl Sephadex A-50 and Sephadax G-200. The purified toxin had a specific toxicity of 5.8×10^7 minimum lethal doses per mg protein and showed 950-fold of that of the culture supernatant.

The purified toxin had a molecular weight of 140,000 and consisted of a heavy and a light chain with molecular weights of 85,000 and 55,000, respectively, which were linked by one disulfide bond. The purified two components had different amino acid compositions and antigenicities. Molecular weights and amino acid compositions of the whole molecule, heavy chain and light chain were very similar to those of type C_1 toxin.

Eight kinds of monoclonal antibodies against D_{1873} toxin were prepared, six of which reacted with the heavy chain, and the other two of which reacted with the light chain. Five kinds of the former neutralized the toxicity of D_{1873} toxin. One of the antibodies acting on the heavy chain and the two acting on the light chain crossreacted with the heavy and light chains of type C_1 toxin, respectively. And the former neutralized also the toxicity of type C_1 toxin.

These results indicate that type D and C_1 toxins are similar in molecular construction with hetero-subunits linked by one disulfide bond, and that D_{1873} and type C_1 toxins have common antigenic regions on the heavy and light chains. The common site on the heavy chains seems to be essential for the actions of the tow toxins.