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Crystal Structure of Clostridium Botulinum Neurotoxin B: The Effect of Reducing the Interchain Disulfide Bond on the Conformation
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Beamline(s): X25

Introduction: The toxigenic strains of Clostridium botulinum produce seven serologically distinct types of neurotoxins labeled A - G (EC 3.4.24.69), while Clostridium tetani produces tetanus neurotoxin (EC 3.4.24.68). Botulinum and tetanus neurotoxins (BoNTs and TeNT) are produced as single inactive chains of molecular mass of approximately 150 kDa. Most of these neurotoxins are released after being cleaved into two chains, a heavy chain (H) of 100 kDa and a light chain (L) of 50 kDa held together by an interchain disulfide bond, by tissue proteinases. BoNT/E is released as a single chain but cleaved by host proteinases (1). Botulinum neurotoxins are responsible for neuroparalytic syndromes of botulism characterized by serious neurological disorders and flaccid paralysis. BoNTs block the release of neurotransmitters like glycine and γ-aminobutyric acid (GABA) in the inhibitory interneurons of the spinal cord resulting in spastic paralysis. In spite of different clinical symptoms, their etiological agents intoxicate neuronal cells in the same way and these toxins have similar structural organization (2).

The intoxication by neurotoxins is proposed to be a four-step process (3, 4-6): 1. cell binding, 2. internalization, 3. translocation into cytosol and 4. enzymatic modification of a cytosolic target. These neurotoxins have been classified as zinc endopeptidases because of the presence of a conserved zinc binding motif, HEExxH+E, approximately in the middle of the light chain (7, 8). The inhibition of exocytosis by neurotoxins in cytosol has been identified as a zinc-dependent specific proteolysis of components in the neuroexocytosis apparatus. They act specifically on protein components of the same neuroexocytosis apparatus present in cytosol.

Reduction of the interchain disulfide is a prerequisite for toxic activity and the rate-limiting step in toxicity (9). The absence of the toxic activity in the unreduced state suggests that after reduction, the toxin undergoes some structural change in order for the substrate to come closer to the active site, which is in a deep cavity. It is also believed that the light chain will separate from the heavy chain to enter the cytosol to attack the targets. We had earlier determined the crystal structure of botulinum type B in the unreduced form. Here we are reporting the structure determination of the protein crystallized under reducing condition.

Methods and Materials: The crystallization condition is similar to the published procedure except that the protein was treated with 10 mM DTT to reduce the inter-chain disulfide bond and the crystallization was done under reducing conditions. X-ray diffraction data were collected at the NSLS Beamline X25 and the data extend to 1.9 Å resolution. The data were processed with Denzo/Scalepack and the crystal structure was determined by the molecular replacement method. The refinement was done with CNS and the final R and R-free are 0.21 and 0.25, respectively.

Results: Except for some minor changes in the loop regions, the two chains stay together in spite of the absence of the interchain disulfide bond. The distance between Sγ - Sγ is more than 3.1 Å and the two chains stay together in a compact form. Also, there is no significant difference in the main chain fold of the molecule.

Conclusions: We believe that reduction of disulfide alone is not enough for the catalytic domain to separate. There could still be an unidentified process required to separate the catalytic domain.

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References: