

Crystal Structure of *Clostridium botulinum* Neurotoxin B in Complex With a Trisaccharide

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The first step in the intoxication by neurotoxins is cell binding. Neurotoxins bind first to the large negatively charged surface of the presynaptic membrane which consists of polysialogangliosides and other acidic lipids. Binding studies have revealed that neurotoxins bind to di and trisialogangliosides like GD1a, GT1b and GD1b, especially the 1b series with an affinity less than 40%. However, in order to produce the level of intoxication by such minute concentrations of toxins (sub-picomolar) the binding affinity to receptors must be very high. Therefore, a double receptor model for binding has been proposed. The toxin binds to the negatively-charged surface of presynaptic membranes through low affinity, high concentration polysialogangliosides and then moves laterally to bind to a yet unknown specific protein receptor. Since the final binding constant is the product of these two binding constants, a very high affinity occurs. Recently while a double receptor model was being tested, a 58 kDa protein from rat brain synaptosomes was shown to bind to BoNT/B, only in the presence of GT1b or GD1a.

In order to study the binding site of BoNT/B and its interaction with gangliosides, the structure of the complex of BoNT/B with sialyllactose was determined to a resolution of 2.6 Å. 3' - sialyllactose with its structural formula, α -Neu5Ac-[2→3]- β -D-Gal-[1→4]-D-Glc partly mimics one branch of the sugar moiety of GT1b which binds to BoNT/B. Sialyllactose was chosen for the soaking study since it is soluble in water unlike GT1b and has a terminal sialic acid which was predicted to bind to BoNT/B. Crystals of BoNT/B were soaked in a mother liquor containing 25 mM of sialyllactose for over a week. Data were collected at X25 beam line of the NSLS to 2.6 Å resolution. Since the R_{merge} between this data set and the native data set was 0.13, BoNT/B model obtained from the native crystals was used to refine the structure. After rigid body and simulated annealing refinement, the R-factor dropped to 0.22. The $F_o - F_c$ map calculated without including the trisaccharide molecule showed clear density near the Trp 1261. The trisaccharide molecule could be modeled in this difference density map. Data were collected at X25 beam line of the NSLS to 2.6 Å resolution. Since the R_{merge} between this data set and the native data set was 0.13, BoNT/B model obtained from the native crystals was used to refine the structure. After rigid body and simulated annealing refinement, the R-factor dropped to 0.22. A difference Fourier at this stage revealed density for the trisaccharide. Sialyllactose was built into the density and model was further refined and the final R-factor is 0.21.

The structure of the BoNT/B:sialyllactose complex provides a model for the interaction for the sugar moiety of ganglioside GT1b and the binding domain. This defines the residues interacting with the sugar moiety and will probably be the same for all botulinum neurotoxins. Since binding of ganglioside to the neuronal cells is the first step in toxicity by neurotoxin, the binding site offers itself as a target for the inactivated recombinant vaccine development. Even though inactivated BoNT's are used as a pentavalent toxoid, there is no recombinant vaccine available at this time. Our model has identified residues involved in the binding site and suggest the residues to be mutated to develop a recombinant vaccine.

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