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A Novel Mechanism for *Clostridium Botulinum* Neurotoxin Inhibition

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Treatment of botulism, an acute paralytic disease caused by the bacterium Clostridium botulinum, is currently only preventive. An experimental vaccine is available, but no drug has been developed yet. Biologists at Brookhaven National Laboratory are studying how a chemical called bis(5-amidino-2-benzimidazolyl)methane (BABIM) inhibits one of the toxins produced by the bacterium. The results of this study could help design new drugs against botulism.

The bacterium *Clostridium botulinum* produces one of the most potent neurotoxins – poisonous proteins acting on the nervous system – to humans. Seven different types of neurotoxins, labeled A to G, are produced by the bacterium.

To infect a neuronal cell, a neurotoxin first binds to the membrane of the cell. Then, the cell membrane curves inward, incorporating the toxin into a vesicle that drifts inside the cell. The toxin escapes the vesicle by creating a channel in the vesicle membrane, inserting its light chain (LC) through the channel, and slipping away completely into the cell's cytosol (fluid portion of the cytoplasm). The toxin is "translocated" from the vesicle to the cytosol. Then, the toxin cleaves specific targets in the cytosol and blocks the release of neurotransmitters – chemicals produced by neurons to communicate with each other – thereby causing muscular paralysis and the eventual death of the patient infected with the bacterium.

The three-dimensional structure of a *botulinum* toxin reveals three domains, called binding, translocation, and catalytic domains, corresponding to the three functions of the toxins.

All seven toxins contain a zinc-based structure in their catalytic domain, but structural details may be different, because each toxin cleaves different types of targets. For example, the catalytic zinc is located in a deep cavity in the active site of all toxins, but the cavity is partially covered by a "belt" region in *botulinum* neurotoxin A (BoNT/A) while it is open in BoNT/B.

Treatment of botulism is currently only preventive: An experimental vaccine is available, but no drug has been developed yet. Therapeutic treatment could be effective at any one of the three stages of toxicity – binding of toxin, internalization, or catalytic activity.

Chemicals such as Bis(5-amidino-2-benzimidazolyl)methane (BABIM) are known to combine with the catalytic zinc, thus preventing the toxin from binding to specific targets, also called substrates, in the cytosol. But the effectiveness of BABIM on the toxin needs to be further investigated.

Authors of the study (left to right): Subramaniam Eswaramoorthy (lead author), Subramanyam Swaminathan, and Desigan Kumaran.

We have determined the crystal structure of a complex of BoNT/B and BABIM, and investigated how BABIM binds to the zinc atom and inhibits the toxin.

The crystal structure of the complex revealed a tunnel that connects a cleft formed between the translocation domain and the catalytic domain to the active site cavity, as shown

in figure 1. Interestingly, two molecules of BABIM bind to the toxin on either side of an aspartic acid molecule, suggesting that the inhibitor can enter and bind to the active site in two different ways. We suggest that one inhibitor molecule has entered through the cleft between the translocation and catalytic domains while the other has entered through the wide opening of the active site cavity.

The inhibitor molecule near the active site perturbs and disrupts the zinc bonds to surrounding molecules in the toxin, as shown in figure 2.

The structure of the BoNT/B-BABIM complex has shown that the active site residues rearrange in the presence of the inhibitor, allowing it to partly occupy the site where the substrate would bind. Also, the zinc atom is progressively removed from the active site and transported to a different site in the protein. We have also shown that it is possible for appropriate inhibitors to enter the active site of the toxin contrary to the belief that the belt surrounding the catalytic domain shields the active site. But the inhibition could be due to either the non-availability of substrate-binding sites, the removal of the zinc atom, or a combination of both.

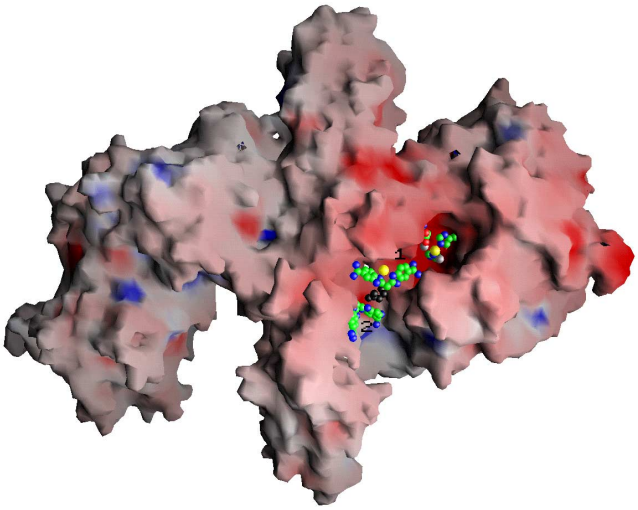

Figure 1. *Representation of the electrostatic potential surface of* botulinum *neurotoxin B. The two molecules of bis(5-amidino-2-benzimidazolyl)methane that are trapped in the tunnel and the residues coordinating the zinc atom are shown as sphere model. Zinc atoms are shown in yellow while water molecules coordinating with the zinc atoms are in silver gray.*

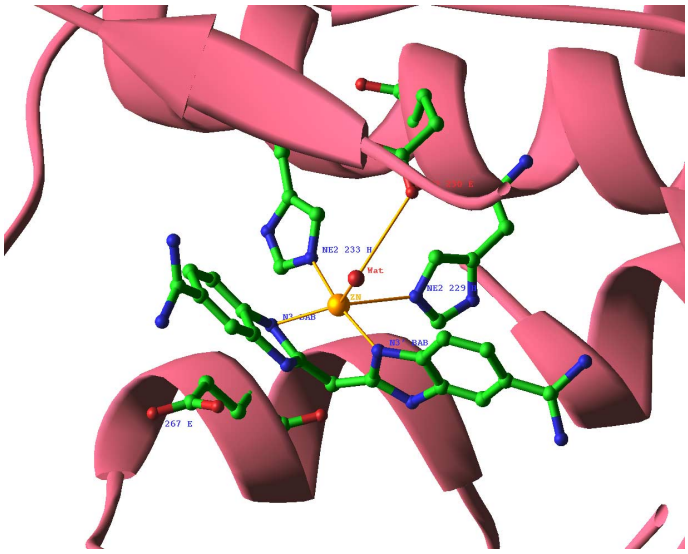

Figure 2*. Representation of bis(5-amidino-2-benzimidazolyl)methane (BABIM) at the neurotoxin's active site, with the light chain and the belt region. Coordination to the zinc atom from protein ligands and BABIM are shown as thin yellow lines.*