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## Botulism Toxin Ensnares Its Target

The first detailed structure of a botulism toxin attached to its target protein reveals that the toxin snakes the protein around itself—a sort of “reverse anaconda”—to recognize the receptor. The new studies show how the toxins that cause botulism and tetanus can recognize and attack particular nerve cell proteins at the neuromuscular junction, which leads to paralysis.

The researchers said their findings could lead to new knowledge that could speed the development of drugs to block botulism or tetanus toxins more rapidly in cases that have progressed beyond the stage at which antibiotics are effective.

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Howard Hughes Medical Institute investigator Axel T. Brunger and graduate student Mark Breidenbach at Stanford University reported their findings December 12, 2004, in an advance online publication in the journal *Nature*.

The neurotoxins from bacteria that cause the paralysis associated with both botulism and tetanus contain enzymes called proteases that cleave specific nerve cell proteins. The nerve cell proteins are called SNAREs, which are key components of the machinery that nerve cells use to fire bursts of neurotransmitter chemicals to trigger neighboring nerves or activate muscle cells. Without SNAREs, nerve function is blocked.

Neurotoxin proteases that act by cleaving SNARE proteins are highly specific for their targets - meaning that each toxin specifically recognizes and attacks one of three different neuronal SNARE proteins. Since most of these

toxin proteases have virtually the same structures at the regions that perform the cleavage, or active sites, a key question has been how they recognize their particular targets, Brunger said.

What was known previously is that other regions of the neurotoxin protein, which they called “exosites,” might be involved in target recognition. However, the location and shape of these exosites were unknown. To search for the location of the exosites, Breidenbach created crystals of a particular botulinum neurotoxin bound to its target SNARE. The researchers determined the structure of the bound proteins using x-ray diffraction, a widely used analytical technique whereby beams of x-rays are directed through crystallized proteins. The resulting pattern of diffraction is analyzed to deduce the protein's atomic structure.

Their structural analysis revealed that the neurotoxin wraps itself in a segment of the SNARE protein by attaching at numerous exosites. This interaction enables the toxin to recognize the SNARE protein with high specificity, said Brunger.

“Our structure has shown for the first time that it's a very extensive interaction on the protein surface, far from the active site, that actually determines specificity,” said Brunger. “This extensive interaction is very unusual for a protease. Up to this point, it's the largest known interface area for such a complex, with numerous points of contact.”

Another notable finding, said Brunger, was that binding of the toxin to its target causes significant conformational change of the enzyme, which quite likely activates its ability to cleave the protein.

When the researchers compared the amino acid sequences of the type of botulinum toxin they studied with other known toxin sequences, they found that the region they had found to contact the SNARE was variable. They theorize that such differences among toxins give them their specificity for their target SNAREs; among toxins that recognize the same SNARE, amino acid variations in the contact region might allow each to cleave its target at different sites.

According to Brunger, the discovery of the mechanism by which the toxins recognize their target proteins could provide new information that will aid in developing drugs that can block the toxins more quickly. “Finding these remote exosites suggests the possibility of developing drugs that could compete with the toxin for an exosite and disrupt the protease's ability to attack its target,” he said. “Such a drug would act instantaneously once it crossed into the cell. And it would not interfere with other essential similar proteases in the cell, because it wouldn't be attacking the active site of the enzyme itself.”

While this kind of drug could prove useful in treating late-stage botulism or tetanus, noted Brunger, effective antibiotics and vaccines already exist to treat the diseases in most cases, if they are caught early.

In further studies, the researchers will extend their analysis to tetanus toxins, and to the other types of botulinum toxins. Such studies, he said, could reveal differences in how the toxins recognize their targets.

“These bacteria have developed very clever enzymatic machines for recognizing proteins, and it may be possible, given our structural knowledge, to modify these proteases for clinical use,” Brunger said. “An intriguing possibility would be to use their specificity as the basis for enzymes engineered to attack proteins involved in disease”.