Drug Discovery & Development

Rockefeller Researchers Solve Structure for Deadly Bacterial Toxin

by Aaron Sender | Jul 01 '04

Researchers at Rockefeller University, New York, have determined the structure of a potent DNA-damaging protein involved in many bacterial diseases. Known as cytolethal distending toxin (CDT), the protein poison is the chemical culprit responsible for leading killer diseases in the developing world, such as typhoid fever and diarrhea. It is the only bacterial toxin known to attack its victim's DNA, and therefore is also a suspected carcinogen.

Studying the structure "is going to help us understand how it works in cells and its role in disease," says principal investigator C. Erec Stebbins, PhD, who heads the laboratory of structural microbiology at Rockefeller University. "We see exactly what the active site looks like," he says. Therefore, a single small-molecule drug that blocks the CDT-active site could conceivably treat a whole range of diseases caused by the same toxin. Stebbins and colleagues describe the protein structure and discuss its function in the May 27 issue of the journal *Nature* . [Stebbins *et al.* , vol. 429, pp. 429-433 (2004)]

They chose protein from *Hemophilus ducreyi* , a bacterium that causes a sexually-transmitted disease called chancroid, for a purely pragmatic reason: "It's the only one that crystallized at all," says Stebbins. In fact, he considers getting proteins to form the crystals needed for protein structure determination the hardest part of the process. "Crystallization is still somewhat of an art. There's no rational method," he says. "We really don't understand a lot about how to predict a priori, how a protein might pack into a crystal and under what conditions. It's completely trial and error at this point," says Stebbins.

The researchers then used X-ray crystallography to solve the structure, a method that uses X-rays that bounce off the electrons of the target protein crystal for indirect clues of molecular shape. "Crystallography is like getting the schematics for a car and the engine," says Stebbins. "If that's all you have, you still have to know a lot more about what cars do, how they interact, and what they are used for to understand what you're looking at."

The structure has provided some insight into how the toxin does its damage. Stebbins confirmed that CDT is made up of three subunits, including CdtB with its DNA-cutting active site. The other subunits, CdtA and CdtC combine with the CdtB and help deliver it into the cell.

Biochemical evidence had previously suggested that CdtA and CdtC work together. But the three-dimensional structure proves it, says Stebbins. "There's a big groove that looks like a valley on top" where the two subunits bind each other, he says. "But part of that groove is CdtA and the other part is CdtB." This strongly suggests that they work together for a single purpose. "Just like any machine, proteins are built to specification. You usually don't see something massive like that in a protein structure unless it's doing something," Stebbins says. He suspects the groove docks with a cell surface protein.

Another striking feature is a large cluster of aromatic, or ring-shaped, molecules on the surface of CdtA. "That's not a typical surface feature in a protein," Stebbins says. "In fact it was the first thing I actually noticed in the structure." It turns out this aromatic batch is well conserved across the CDT-producing species. To examine the function of this patch, the Stebbins lab created proteins in which that area is mutated. They found that this does not stop the assembly of the protein, but it did negate the activity of the toxin. This suggests that the aromatic batch is part of the machinery that allows the DNA-destroying CdtB subunit to invade the cell.

The A and C units are also very similar to domains in the ricin toxin, but the most highly conserved part of the protein is the active site of CdtB. "We conclusively showed that it belongs to a certain nuclease family." Those enzymes are known for their DNA-chopping activity, says Stebbins.