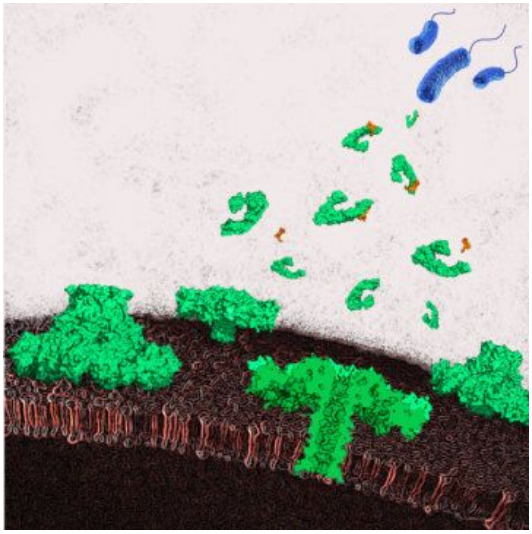
A Toxic Quick-Change Artist

Date: July 13, 2016

Source: University of Bern

Summary: Molecular biologists have discovered a mechanism which enables a deadly toxin to penetrate and destroy human cells. Their findings can serve a rational framework for the design and development of new anti-toxin drugs.

FULL STORY



Aeromonas hydrophila pathogenic bacteria (blue) secrete aerolysin toxin (green). At the surface of its target cell, the protein undergoes major changes that lead to the formation of a pore in the host cell plasma membrane. The host cell thereby gets leaky and dies.

Credit: © Nuria Cirauqui, Universidade Federal do Rio de Janeiro.

Molecular biologists at the University of Bern have discovered a mechanism which enables a deadly toxin to penetrate and destroy human cells. Their findings can serve a rational framework for the design and development of new anti-toxin drugs.

Pathogenic bacteria produce a variety of toxins in order to attack their hosts. Some of these toxins have also been classified as potential bioterrorism weapons. A particularly efficient and deadly type of toxin punches holes in the membrane of host cells, and thereby kills them. This type of toxin, called pore forming toxins, is found in a very large number of bacteria.

Aeromonas hydrophila is a bacterium that produces a pore forming toxin called aerolysin. By killing cells that line the gut of their host or that are exposed at the surface of an open wound, aerolysin helps the bacterium to feed on the released cell

content and to penetrate deeper in the human body. As a consequence of gut cell death, the patient suffers of severe diarrhea. A. hydrophila can also cause deep wound infections and sepsis in humans.

In addition to their pathogenic function, aerolysin, and the majority of pore forming toxins, are most interesting because of an unusual duality: they are secreted by the bacterium as fully water-soluble proteins, which then assemble and insert into the host cell membrane to become genuine transmembrane proteins. The mechanism by which aerolysin transition from this water-soluble to the membrane-inserted state is not well understood and constitutes an active area of research.

Using state of the art electron microscopy technology and several designed aerolysin mutants an international group of molecular biologists led by the University of Bern and with participation of the EPFL (both Switzerland) have obtained the atomic structure of aerolysin prior, during, and after membrane insertion. Their results significantly improve the understanding of the function of a major class of toxins. The study is now being published in Nature Communications.

Four steps to kill the cell

"Aerolysin is a toxin with an extreme stability," says Benoît Zuber, who led the projects together with Ioan Iacovache, both from the institute of Anatomy at the University of Bern. "This is due to its novel protein core design which we termed double concentric beta-barrel." In a first step, the water-soluble toxin assembles at the target cell surface around this core design, which makes the cell unable to repair the damage resulting from aerolysin pore formation. "That's why aerolysin-like pore forming toxins are of the most potent pore forming toxins known," says Zuber. In a second step, a part of the protein rearranges to form a molecular bow and arrow. Then the toxin undergoes a large collapse, which shoots the arrow into the target cell membrane, resulting in the formation of a molecular channel through the membrane. Finally, the tip of the arrow bends outward to form a hook and thereby firmly anchors the toxin in the membrane.

"Aerolysin can transform itself like a quick-change artist," says Zuber. "Understanding these changes should be very useful to design new and more potent drugs against diseases resulting from Aeromonas infection," adds Iacovache.

Important insights for DNA research and the formation of diseases

Furthermore, pioneering nanotechnology research from other groups has demonstrated that aerolysin could be used as a very powerful tool to sequence DNA. The detailed knowledge of aerolysin structure will enable fine tuning of the pore property for this purpose. Finally, the novel double concentric beta-barrel corresponds to a hypothetic structure adopted under some conditions by beta-amyloid peptides, the causative agents of Alzheimer's disease.

Visualizing proteins at atomic resolution

The introduction of a new generation of digital camera for electron microscopes, the so-called direct electron detectors, four years ago has enabled scientists to visualize the structure of proteins and determine the position of the atoms composing these proteins. Previously only two methods, X-ray crystallography and nuclear magnetic resonance, could provide such level of details. The former method necessitates

however having crystals of the investigated protein, which in the case of transmembrane proteins has been extremely challenging. The latter method is restricted to very small proteins.

"The new electron microscopy methodology is a major breakthrough in biomedical sciences as it has enabled the discovery of the atomic structure of a whole new set of proteins" says Zuber. This has been recognized by the prestigious journal Nature Methods, which elected the so-called single particle cryo-electron microscopy method of the year 2015.

Journal Reference:

1. Ioan Iacovache, Sacha De Carlo, Nuria Cirauqui, Matteo Dal Peraro, F. Gisou van der Goot, Benoît Zuber. **Cryo-EM structure of aerolysin variants reveals a novel protein fold and the pore-formation process**. *Nature Communications*, 2016; 7: 12062 DOI: 10.1038/NCOMMS12062