Scientists reveal structure of dangerous bacteria's powerful multidrug resistance pump

September 22 2010

A team at The Scripps Research Institute has detailed the structure of a member of the only remaining class of multidrug resistance transporters left to be described. The work has implications for combating dangerous antibiotic resistant strains of bacteria, as well as for developing hardy strains of agricultural crops.

The study was published in an advance, online issue of the journal *Nature* on September 22, 2010.

"Now with our crystal structure, scientists can for the first time figure out exactly how this transporter works," said the study's senior investigator, Geoffrey Chang, Ph.D., associate professor in the Scripps Research Department of Molecular Biology. "This could lead to the design of drugs that evade or inhibit the transporter, or to reengineering the transporter to help some plants grow in soil they can't grow in now."

The protein described in the study, NorM, was found in the virulent bacteria *Vibrio cholerae*. *V. cholerae* causes cholera, a disease that affects the small intestine and is a common cause of death in developing nations. The NorM transporter is responsible for widespread resistance to ciprofloxacin and other fluoroquinolones (a broad-spectrum, inexpensive class of antibiotics) and to tigecycline, a new class of drug specifically designed to overcome that antibiotic resistance.

Importantly, NorM is a member of the multidrug and toxic compound extrusion (MATE) family that is involved in important biological functions across all kingdoms of life. These transporters defend plant, animal, and microbial cells by pumping out toxic chemicals before they can have any effect. In addition to antibiotic resistance, MATE transporters are associated with resistance to a commonly used diabetes drug, as well as resistance to anti-inflammatory and anti- arrhythmia agents. In plants, MATE transporters help to neutralize the acidity of soil, directly affecting crop yields worldwide.

"By showing how a key member of the [MATE transporter] family undergoes shape changes during the extrusion process, this work may lead to new ways to block the transporter, with possible applications in medicine and agriculture," said Jean Chin, Ph.D., who oversees this and other structural biology grants at the National Institutes of Health (NIH).

"Herculean Effort"

It took a "Herculean effort" to produce the high-resolution crystal structure of NorM, Chang noted. The researchers found it was difficult to produce enough protein to work with, and hard to purify the transporter in its natural state.

After the team found a way to produce and purify the protein, the scientists still needed to create crystals to be able to use a technique known as x-ray crystallography to solve its structure. In this method, scientists produce and purify large quantities of a protein that are crystallized. The crystal is then placed in front of a beam of x-rays, which diffract when they strike the atoms in the crystal. Based on the pattern of diffraction, scientists can reconstruct the shape of the original molecule. In this case, though, the NorM crystals were unusually fragile under an x-ray beam.

After many attempts, however, the research team succeeded in producing two crystal structures of the NorM transporter as it sat on the outside surface of *V. cholerae*. One showed the transporter by itself and the other provided a snapshot of how the pump is powered by sodium ions.

The NorM transporter normally sits, waiting, on the inside of the bacterial cell membrane for toxic chemicals—in this case antibiotics—that seep inside. The protein then changes shape in order to scoop the chemical up, and transport it back through the cell wall to the outside of the bacteria, keeping the bacteria safe from destruction.

The structure of this bacterial pump revealed a shape distinct from all other MDR transporter families, say co-authors Xiao He and Paul Szewczyk, graduate students at the University of San Diego, California, (UCSD) who worked with Chang to derive the structure. The pair also took the lead in the effort to verify the crystal structure - a process of labeling 16 different amino acids on the protein and confirming their three-dimensional position. This part of the effort took 18 months.

On the outside of the bacteria, the transporter looks like an upside down "V" shaped lampshade, He said, and the chemical to be removed presumably fits inside the narrow part of the structure. She adds that the research team is working to crystallize the transporter on the inside of the bacterium, as well as the structure with a chemical bound to it.

"Bacteria have a number of different transporter systems, so it is important to design antibiotics that will not be instantly pumped out," He noted.

With the atomic structure of NorM solved, the team continues to investigate other MATE transporters, including those found in plants and those that exist in human liver and kidney cells that can reduce the

effectiveness of a wide variety of drugs.

Provided by The Scripps Research Institute