Scientists crack code of critical bacterial defense mechanism

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Scientists have combined chemistry and biology research techniques to explain how certain bacteria grow structures on their surfaces that allow them to simultaneously cause illness and protect themselves from the body's defenses.

The researchers are the first to reproduce a specific component of this natural process in a test tube - an essential step to fully understanding how these structures grow.

With the new method described, these and other researchers now can delve even deeper into the various interactions that must occur for these structures - called lipopolysaccharides - to form, potentially discovering new antibiotic targets along the way.

Lipopolysaccharides are composed primarily of polysaccharides - strings of sugars that are attached to bacterial cell surfaces. They help bacteria hide from the immune system and also serve as identifiers of a given type of bacteria, making them attractive targets for drugs. But before a drug can be designed to inhibit their growth, scientists must first understand how polysaccharides are developed in the first place.

"We were able to answer some of the questions about how components of this growth system do their jobs. This will allow us to more fully characterize lipopolysaccharide biosynthesis in vitro, a process which may shed light on useful targets for developing antibiotic agents," said Robert Woodward, a graduate student in chemistry at Ohio State

University and lead author of the study.

The study is published in the April 25 online edition of the journal *Nature Chemical Biology*.

The researchers used a harmless strain of Escherichia coli as a model for this work, which would apply to other E. coli strains and similar Gram-negative bacteria, a reference to how their cell walls are structured.

The surface of these bacteria house the lipopolysaccharide, which is a three-part molecular structure embedded into the cell membrane. Two sections of this structure are well understood, but the third, called the O-polysaccharide, has to date been impossible to reproduce.

Two significant challenges have hindered research efforts in this area: The five sugars strung together to compose this section of the molecule are difficult to chemically prepare in the lab, and one of the key enzymes that initiates the structure's growth process doesn't easily function in a water-based solution in a test tube.

Ohio State synthetic chemists and biochemists put their heads together to solve these two problems, Woodward said.

To produce the five-sugar chain, the researchers started with a chemically prepared building block containing a single sugar and introduced enzymes that generated a five-sugar unit from that single carbohydrate.

"The first part was done chemically, and in the second part, we used the exact same enzymes that are normally present in a bacterial cell to transform the single sugar into a five-sugar string," Woodward said.

Once these sugars join to make a five-sugar chain, a specific number of

these chains are joined together to fully form the O-polysaccharide. A protein is required to connect those chains - the protein that doesn't respond well to the test-tube environment.

Early attempts to produce this protein in the lab resulted in clumping structures that did not function. So Woodward and colleagues produced this protein in the presence of what are known as "chaperone" proteins.

"And basically what the chaperones do is help the protein fold into its correct state. We were able to produce the desired enzyme and also were able to verify that it was functional," Woodward said.

This protein is called Wzy. It is a sugar polymerase, or an enzyme that interacts with the five-sugar chain to begin the process of linking several five-sugar units together.

Getting this far into the process was important, but the researchers also completed one additional step to define yet another protein's role.

Wzy connected the five-sugar chains, but it did so with no defined limit to the number of five-sugar units involved, a feature that does not match the natural process. On an actual bacterial cell wall, the length of the polysaccharide falls within a relatively narrow range of the number of chains connected.

So the scientists introduced another protein, called Wzz, to the mixture. This protein is known as a "chain length regulator." With this protein in the mix, the lengths of the resulting polysaccharides were confined to a much more narrow range.

"We were able to replicate the exact polysaccharide biosynthetic pathway in vitro, getting the correct lengths," Woodward said. "This is important because now you can begin to look at a whole host of other

properties in the system."

The group already started trying to answer one compelling question: whether the two proteins, Wzy and Wzz, have to interact to fully achieve formation of the polysaccharide.

"We've shown in some preliminary results that they do interact, but we haven't determined whether that interaction has any functional relevance," Woodward said.

With this knowledge in hand, researchers now have access to information about how all three parts of the lipopolysaccharide, the large biomolecule on Gram-negative bacteria cell surfaces, is formed. One thing they already knew is that the entire process takes place on an inner membrane and is then exported to the outer membrane on the cell surface.

Now that scientists can reproduce formation of the lipopolysaccharide, they can more directly characterize the export process - a step in the pathway that serves as another potential antibiotic target, Woodward noted.

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