Moonlighting proteins can make bacteria pathogenic

Proteins either remain in a cell or leave it, and which they do depends on their function. However, the mechanism underlying the export of biomolecules from cells remains unknown. Prof. Dr. Friedrich Götz and his team of researchers at the Institute of Microbial Genetics at the University of Tübingen have found out that staphylococci can turn into dangerous pathogens by excreting normally harmless enzymes. The researchers believe that the enigmatic excretion of such enzymes is due to a completely new mechanism and are thus planning to carry out further studies to explore how proteins leave cells. The researchers' overall goal is to develop a system for identifying compounds that can impair enzyme excretion and thus counteract the bacteria's virulence.

Prof. Dr. Friedrich Götz is the director of the Department of Microbial Genetics at the Interfaculty Institute of Microbiology and Infection Medicine (IMIT) at the University of Tübingen. © University of Tübingen

Proteins are produced inside cells and then labelled with signals that determine their subsequent use and location. Some signals indicate incorporation into the cell membrane while others indicate the excretion of the labelled protein from the cell. Enzymes that are part of the normal cell metabolism remain in the cell and are not labelled. Nevertheless, scientists have observed that such enzymes are sometimes also exported from both bacterial and mammalian cells. How and why these cytoplasmic proteins are exported is not yet known.

Prof. Friedrich Götz and his team of scientists at the Institute of Microbial Genetics and Infection Medicine at the University of Tübingen have been working for a while to elucidate the mechanisms used by enzymes without special export signals to leave the cell and why they are actually excreted. These investigations are part of the DFG-funded CRC 766 project entitled "The Bacterial Cell Envelope". "We have observed that cytoplasmic proteins are not always excreted in the classical way from bacterial cells," says Prof. Götz. "We initially assumed that the proteins leave the cell by way of cell lysis, phages or as a result of age. However, our findings have shown that it is not as simple as it

seems. We assume that the cells use a specific secretion mechanism. And we also assume that this mechanism is used by several groups of bacteria. It might even be found in all bacteria."

Harmless metabolic enzymes become dangerous

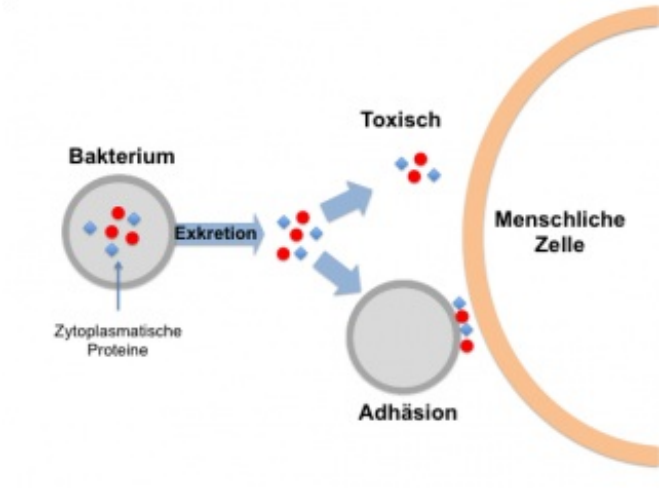
The microbiologists from Tübingen are specifically focused on Staphylococcus aureus – a common bacterium that is normally harmless but can sometimes cause disease, including a number of inflammatory diseases that might lead to life-threatening complications. MRSA (methicillin-resistant Staphylococcus aureus) has become a major problem in clinical medicine. MRSA is resistant to almost all existing antibiotics. In their attempt to identify the function of cytoplasmic MRSA proteins outside the cell, the researchers found that bacterial culture supernatant frequently contained fructose-1,6-bisphosphate-aldolase (FbaA) and glyceraldehyde-phosphate dehydrogenase (GAPDH), two enzymes that play a key role in the carbohydrate metabolism.

M.Sc. Patrick Ebner and his colleagues study the mechanisms underlying the bacterial excretion of proteins and have come up with interesting results. © University of Tübingen

"We therefore assumed that the two excreted enzymes must give the pathogens an advantage in terms of infection," said Götz. "When we took a closer look, we found that the two normally harmless cytoplasmic enzymes actually had a cytotoxic effect on human cells as soon as they had left the bacterial cell. And not only that, our binding studies demonstrated that both enzymes bound to certain host matrix proteins and enhanced adherence of bacterial cells to host cells." The microbiologists then injected both enzymes into wax moth larvae and observed that far fewer larvae survived than when control proteins were injected into them. "For us, this was clear evidence of the proteins' cytotoxic activity," said Götz. "We now intend to study this cytotoxic activity in greater detail."

Moonlighting proteins – different place, different activity

The two enzymes, FbaA and GAPDH, are normally responsible for the breakdown of sugar during glycolysis in bacterial cells, and are not harmful. However, they lead to the death of the host cell when they are excreted from the bacterial cell. "The name for molecules that exert different functions at different locations is moonlighting proteins," says Götz. This activity appears to play a key role in the virulence of pathogenic staphylococci as well as other pathogenic bacteria. "We have observed similar things in experiments with streptococci, which are bacteria that cause scarlet fever and other illnesses," said Götz. "The phenomenon is not only found in one pathogenic bacterial species. It appears that the pathogenicity of bacteria correlates with the amount of cytoplasmic proteins excreted. The more proteins are excreted the more pathogenic the bacteria are and vice versa. Non-pathogenic bacteria excrete far fewer cytoplasmic proteins" (Ebner, 2016). These findings support the researchers' hypothesis that



Overview of the effect of excreted cytoplasmic proteins: some cytoplasmic proteins, including GAPDH and FbaA, are excreted from bacterial cells by way of an as yet unknown mechanism. The two enzymes play a key role in breaking down glucose (glycolysis). Outside bacterial cells, the two enzymes are toxic for human cells and mediate the adhesion of the bacteria to human cells. Due to this dual function, these proteins are also referred to as "moonlighting proteins" because they exert different functions inside and outside the cell. © University of Tübingen

moonlighting protein excretion is subject to a mechanism that is crucial for the pathogenicity of bacteria and is the reason why some bacterial species, pathogenic staphylococci for example, are more dangerous for humans than others.

Exploring enigmatic excretion of proteins

The microbiologists from Tübingen will now carry out further studies to explore the mechanism underlying the enigmatic excretion of proteins. "As far as we can tell, this mechanism looks to be completely different from known mechanisms," says Götz. The researchers also want to find out why the two glycolytic enzymes become pathogenic outside the cell. One reason might be that moonlighting proteins have a negative effect on the metabolism of the host cell. Another hypothesis is that the pure ability to attach to matrix proteins on the surface of the host cell leads to changes that will eventually damage the cell. The biologists' ultimate goal is to develop a test system to screen a substance library and find substances that could potentially prevent the excretion of cytoplasmic proteins and thus be used as antibiotic agents.

Reference:
Ebner P, Rinker J, Nguyen MT, Popella P, Nega M, Luqman A, Schittek B, Di Marco M, Stevanovic S, Götz F. Excreted cytoplasmic proteins contribute to pathogenicity in Staphylococcus aureus. Infect Immun. 2016 May 24;84(6):1672-81, DOI 10.1128/IAI.00138-16.

Article

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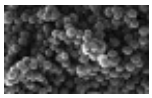

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