Insect gut microbe with a molecular iron reservoir

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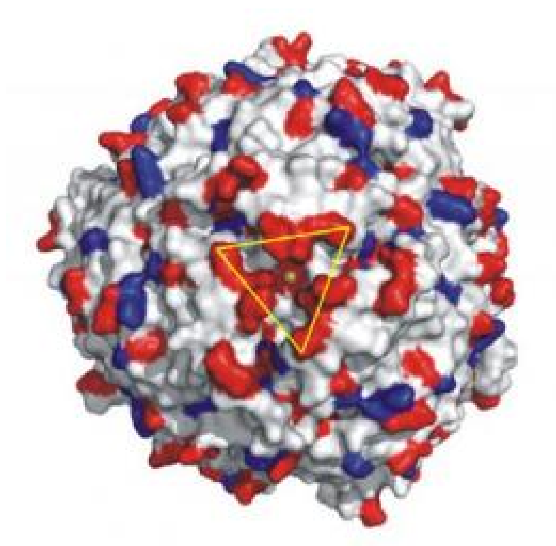


Representation of the protein surface with negative (red) and positive (blue) charges. The triangle marks the iron uptake pore. Credit: Kornelius Zeth, MPI Tuebingen

Microbes are omnipresent on earth. They are found as free-living microorganisms as well as in communities with other higher organisms. Thanks to modern biological techniques we are now able to address the complex communities and study the role of individual microorganisms and enzymes in more detail.

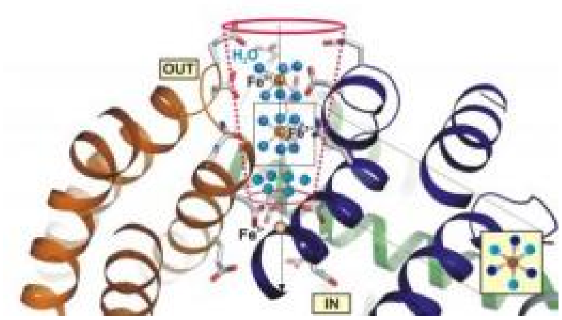
Microbacterium arborescens is a bacterium, which can be found in the guts of herbivorous caterpillars. The Department of Bioorganic

Chemistry at the Max Planck Institute for Chemical Ecology studies interactions between insects and microorganisms which live in their digestive system. What is the advantage for both, insects and microbes? How strongly do they depend on each other? Do microbes play a role in mediating interactions between herbivorous insects and host plants? In the course of the experiments to answer these questions the scientists came across an enzyme they had isolated from *M. arborescens*, a resident in the gut of the Beet Armyworm *Spodoptera exigua*. It was called N-acyl amino acid hydrolase (AAH) because of its catalytic function: it catalyzes the synthesis and hydrolysis of conjugates of the amino acid glutamine with fatty acids. The N-acyl glutamines enter the infested plant via oral secretions and intestinal contents of the larvae and trigger the plant's defense responses.

After cloning and sequencing the AAH encoding gene the scientists discovered an interesting result: AAH is closely related to proteins from other microorganisms: the "DNA protection during starvation (DPS)" proteins, which bind to DNA molecules and protect them by crystallization or by removal of dangerous OH• radicals. Jelena Pesek, PhD student in the Department of Bioorganic Chemistry at the institute, was surprised that the enzyme AAH from *M. arborescens* differs from DPS enzymes in other microbes to the effect that it additionally regulates the concentration of N-acyl glutamine (conjugates of glutamic acid with fatty acids) in the gut, which are important for molecular plant-insect interactions. Moreover, the enzyme is able to store Fe(III)ions in its center. If free Fe(II) is present, hydrogen peroxide (H_2O_2), which is synthesized by the insect's intestinal cells to fend off microorganisms, is converted to highly reactive hydroxyl radicals. The process is known as the Fenton's Reaction:

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet HO$ (Fenton's Reaction)

The highly reactive hydroxyl radical •HO damages especially the DNA and thus causes dangerous mutations of the genetic material. In cooperation with Kornelius Zeth from the Max Planck Institute for Developmental Biology in Tuebingen the researchers succeeded in analyzing the iron transport mechanisms by means of crystallization and X-ray structure determination.



Longitudinal section through the pore along with a representation of the iron uptake mechanism. Entering Fe(II)ions, surrounded by 6 water molecules (spatial representation in the box on the right below), are oxidized to Fe(III)ions with a simultaneous loss of their hydrate shell. The Fe(III) is stored as Fe_2O_3 in the center of the macromolecule. Credit: Kornelius Zeth, MPI Tuebingen

The protein consists of 12 identical subunits and has a molecular mass of 204 kDa - a considerable size for a single enzyme. The homo-oligomer is round and hollow inside. It can store up to 500 iron atoms as ferric iron (usually in the form of Fe_2O_3) in the hollow cavity. The iron transport into the cavity is unique: The spherical protein has four selective pores which provide access only to ferrous iron ions along with their hydration shells (six water molecules). At catalytic ferroxidase centers inside the cavity the Fe(II) is oxidized to Fe(III) with simultaneous reduction of the dangerous H_2O_2 to water (H_2O).

The scientists assume that AAH ensures survival of *M. arborescens* in the larval gut, where conditions may be harsh and constantly changing depending on food quality. The enzyme is protective against oxidative stress, reducing the concentration of free Fe(II) by storing it and simultaneously neutralizing H2O2 as a source for cell damaging radicals. The evolutionary context of these processes as well as the formation and hydrolysis of N-acyl glutamines which are also catalyzed by AAH are still unknown. Because of their detergent character these compounds may help the larvae to better digest the plant food. In the course of evolution, attacked host plants may have "learned" to exploit the conjugates which enter the leaves during herbivory as a chemical alarm signal in order to activate their defense against the insect pest efficiently.

More information: Jelena Pesek, Rita Büchler, Reinhard Albrecht, Wilhelm Boland, Kornelius Zeth: Structure and Mechanism of Iron Translocation by a Dps Protein from Microbacterium arborescens. *The Journal of Biological Chemistry* 286. DOI: 10.1074/jbc.M111.246108

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