Enzyme helps bacteria defend themselves against oxidants secreted by immune system

March 21 2017

A research project conducted at the University of São Paulo (USP) in Brazil in collaboration with other Brazilian research institutions and abroad has revealed new aspects of the action mechanism of organic hydroperoxide resistance (Ohr) enzyme, which enables several species of bacteria to neutralize oxidizing substances released by the defense system of the host organism, be it a plant or an animal.

According to the researchers, the resulting knowledge could be the basis for novel therapeutic approaches.

"There are known proteins with a structure similar to Ohr's in plants and animals. That suggests it's possible to inhibit the enzyme in bacteria without causing significant harm to the infected organism, making it an interesting target for drug development," said Luis Eduardo Soares Netto, a professor at the University of São Paulo's Bioscience Institute (IB-USP) and the principal investigator for the study.

However, he stressed that more research is required to produce data relating the presence of Ohr to pathogen virulence.

Linked to the Center for Research on Redox Processes in Biomedicine (Redoxoma), one of the Research, Innovation and Dissemination Centers (RIDCs) funded by FAPESP, Netto's team performed several experiments, often using pathogens, to understand how Ohr participates in bacterial anti-oxidant defense.

"When we started the research project, we knew Ohr had an anti-oxidant function but knew nothing about the physiological substrates for this enzyme," Netto said. "We show in the study that it most effectively neutralizes peroxides, especially long-chain fatty acid hydroperoxides, and peroxynitrite."

To reach this conclusion, the researchers initially performed molecular docking tests in computer simulations that showed how the possible substrates docked in the Ohr active site. These analyses pointed to significant structural complementarity between Ohr and different types of fatty acid hydroperoxides, such as those derived from arachidonic acid and linoleic acid, which act as mediators of inflammatory processes in mammals and plants, respectively.

This first finding was validated in in vitro biochemical assays with Ohr produced by Xylella fastidiosa, the bacterium that causes citrus variegated chlorosis (CVC), a serious disease of sweet oranges and other citrus species. As Netto explained, this research followed from the X. fastidiosa whole-genome sequencing project completed in the 1990s with FAPESP's support.

The in vitro tests consisted of incubating purified Ohr with various types of hydroperoxide. The aim was to measure the time taken by the enzyme to convert each of these oxidants into less toxic substances.

"We observed, for example, that [Ohr] was able to neutralize hydrogen peroxide but that the process was 100,000 times slower than in the case of arachidonic acid hydroperoxide," Netto said.

The chemical reaction occurred in milliseconds when the enzyme was incubated with fatty acid hydroperoxides, but with other types of hydroperoxide, it took minutes, he explained.

The researchers were surprised to find Ohr acting just as efficiently in contact with peroxynitrite as it did with hydroperoxides derived from arachidonic acid and linoleic acid, given that this was not predicted by the computer simulations.

"Peroxynitrite is the product of two other radicals: superoxide and nitric oxide. It's released by both plants and mammals in response to infection by pathogens," Netto explained.

The next step consisted of microbiological assays using lineages of the bacterium Pseudomonas aeruginosa, which causes opportunistic infections in the respiratory system and elsewhere in humans.

"We compared a group of mutant bacteria, in which the Ohr gene had been deleted, with wild bacteria that produced the enzyme," Netto said. "Both groups were placed in different hydroperoxide concentrations to test their resistance."

The wild bacteria grew even in high hydroperoxide concentrations, while the mutant lineages stopped multiplying even in low concentrations. However, when the Ohr gene was reinserted into the mutant bacteria, their resistance to the oxidant was comparable to that of the wild bacteria.

According to Netto, during their evolution, bacteria developed a vast repertoire of anti-oxidant proteins to circumvent host organisms' defenses.

The tests performed by Netto's group showed that other mutant bacteria, in which the genes for these anti-oxidant enzymes had been deleted, were not as sensitive to fatty acid hydroperoxide and peroxynitrite as the mutant lineage without Ohr. According to Netto, this suggests that Ohr plays a central role in bacterial anti-oxidant defense.

More information: Thiago G. P. Alegria et al. Ohr plays a central role in bacterial responses against fatty acid hydroperoxides and peroxynitrite, *Proceedings of the National Academy of Sciences* (2017). DOI: 10.1073/pnas.1619659114

Provided by FAPESP