Coenzyme rare to bacteria critical to Mycobacterium tuberculosis survival

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Coenzyme F420, a small molecule that helps certain enzymes transfer electrons, is found in microorganisms known as methane-producing archaea, some of which thrive in extreme environments. It also helps the bacterium that causes tuberculosis (TB) to survive the defenses of the human immune system. Scientists have now discovered at least one way F420 helps to arm the pathogen.

The research will appear in the early online issue of the *Proceedings of the National Academy of Science* (*PNAS*) during the week of March 23, 2009, in the article, "Conversion of NO2 to NO by Reduced Coenzyme F420 Protects Mycobacteria from Nitrosative Damage," by Endang Purwantini and Biswarup Mukhopadhyay, both with the Virginia Bioinformatics Institute (VBI) at Virginia Tech.

Mukhopadhyay's lab specializes in the study of the anaerobic archaea, especially those that produce methane, and has a program on enzymes that utilize coenzyme F420.

Coenzyme F420 is rare in bacteria. Only the Actinobacteria, a group of aerobic microorganisms, contain F420. They include the mycobacteria, which generally live in the soil, except for Mycobacterium tuberculosis (Mtb), which causes TB.

In 1996, Purwantini as a graduate student in the laboratory of Lacy Daniels, then at the University of Iowa, discovered an enzyme that reduces F420 by adding two electrons and one proton to it, producing

F420H2. This discovery raised the question, "What is the use of F420H2 in a mycobacterial cell?"

In 2000, the PathoGenesis Corporation developed a new anti-TB drug called PA-824 that is converted into an active form within the Mtb cells. Further research by others showed that this conversion requires F420H2. "But that would hardly seem to be why the bacterium makes F420H2," said Mukhopadhyay, assistant professor with VBI and adjunct assistant professor in the Departments of Biochemistry and Biological Sciences. "The organism must have a use for F420H2 that is advantageous to itself."

To find clues to how mycobacteria use F420H2, Purwantini, by this time a senior scientist at VBI, considered the battle between the human immune system and Mtb. Immune cells called macrophages engulf Mtb cells and bombard the pathogen with oxidizing compounds, such as hydrogen peroxide, superoxide, and nitric oxide (NO). In addition, macrophages convert NO into more deadly nitrogen dioxide (NO_2). Mtb can withstand these attacks. Based on earlier research by others, there were indications that F420 is in some way responsible for this resilience of the Mtb.

Purwantini focused on the defense of Mtb against NO_2 and found that F420H2 reacts with NO_2, converting it into much less harmful NO. Purwantini and Mukhopadhyay theorized the following possibility: As a macrophage generates NO and then converts it to NO_2, Mtb responds by converting NO_2 back to the less toxic NO by using F420H2, buying time until the macrophage dies. Mtb then becomes dormant within the dead macrophage, lurking at the heart of the immune system until the system has a weak moment - perhaps as a result of HIV or poor nutrition.

To support this hypothesis, they conducted tests with Mycobacterium smegmatis, a nonpathogenic cousin of Mtb. Wildtype M. smegmatis

survived almost as well in the presence of NO2 as it did in water. But when the researchers knocked out one of the genes required for the synthesis of F420, the bacterium became very sensitive to NO2. They got the same result by knocking out the gene that coded for the enzyme that produces F420H2. When those genes were restored, M. smegmatis regained its resistance to NO2.

Are there other examples for such a defense system? The team found an answer to that question in the literature. Research in early 1990's showed that gamma-tocopherol, a type of vitamin E that is found in certain food materials, converts NO2 to NO and thereby prevents transformation of normal human cells to malignant tumor cells by NO2.

Purwantini and Mukhopadhyay added that, "We know the biochemistry of F420 really well based on the work on methane-producing archaea by us and others and we were able to apply that knowledge to our work on the mycobacetria. It shows how basic science information from different fields can contribute to each other."

What's next? In the immediate future, Purwantini and Mukhopadhyay want to determine the chemical mechanism of the reaction. In the long-term, they want to develop a way to intervene in F420H2 production in the Mtb cell, which will make the organism more prone to being killed by the human immune system. They also speculate that the reaction that they have found may act as a sensor. Mtb could use this reaction to gauge whether a host is capable of making NO2 and therefore immuno competent. In the absence of this reaction, Mtb could wake up from dormancy and cause active TB. This idea has a parallel in methanogenic archaea where F420 has been proposed as a probe for assessing hydrogen availability.

Lacy Daniels, now professor of pharmaceutical sciences with the Irma Lerma Rangel College of Pharmacy, Texas A&M Health Science

Center, said of Purwantini and Mukhopadhyay's continuation of his early work, "This discovery provides the first solid evidence that F420 is truly important for the ability of Mtb to cause disease. It will clearly stimulate efforts to study the role of F420 in animal disease models, and to study inhibitors of F420 metabolism as potential anti-TB drugs."

Robert White, associate professor of biochemistry at Virginia Tech and an expert on biosynthesis, structure, function, and genetics of the coenzymes, said of the research, "This work establishes a new function for a well studied coenzyme that is known to have only a limited distribution in microorganisms. It represents a fine example of basic scientific research providing leads for new drug targets."

Source: Virginia Tech (news : web)