Metal ions regulate terpenoid metabolism in insects

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This image shows the larvae of horseradish leaf beetle *Phaedon cochleariae*. Credit: Sindy Frick / MPI for Chemical Ecology

Max Planck scientists in Jena, Germany, have discovered an unusual regulation of enzymes that catalyze chain elongation in an important secondary metabolism, the terpenoid pathway. In the horseradish leaf beetle *Phaedon cochleariae* a single enzyme can trigger the production of two completely different substances depending on whether it is regulated by cobalt, manganese or magnesium ions: iridoids, which are defensive substances the larvae use to repel predators, or juvenile hormones, which control insect's development. Insects unlike plants do not have a large arsenal of the proteins called isoprenyl diphosphate synthases. Therefore they may have developed another efficient option to channel metabolites into the different directions of terpenoid metabolism by using metal ions for control.

Natural products: 40,000 terpenes

Apart from the primary metabolism which produces substances that ensure the survival of the cells, there are additional biosynthetic pathways in all organisms. Their products may be less important for a single cell, but they can nevertheless be essential for the whole organism. These pathways are summarized as secondary metabolism. One of them is the terpenoid pathway: with more than 40,000 different known structures it generates one of the largest classes of natural products. Terpenoid molecules have diverse functions and can act as components in molecular signaling pathways, as toxins, fragrances or hormones.

The basic unit of all terpenes is a simple molecule containing five carbon atoms that can be joined to chains of different length. There are monoterpenes (C10 units, 2 x C5), sesquiterpenes (C15, 3 x C5), and even polymers, such as natural rubber, which comprises several hundred C5 units. Special enzymes mediate chain elongation. These enzymes have attracted the curiosity of scientists at the Max Planck Institute for Chemical Ecology, Jena, and the Leibniz Institute for Plant Biochemistry in Halle. They studied mechanistic alternatives of how chain elongation is regulated.

Metal ions instead of specialized enzymes

Enzymes involved in chain elongation belong to the group of isoprenyl diphosphate synthases. Such an enzyme was isolated from larvae of the horseradish leaf beetle *Phaedon cochleariae*. It raised the interest of Antje Burse, project group leader in the Department of Bioorganic Chemistry at the Max Planck Institute for Chemical Ecology.

Experiments with larvae in which the enzyme encoding gene was silenced showed that the protein was involved in the formation of the C10 monoterpene chrysomelidial that larvae produce to defend themselves against predators. The larvae accumulate this monoterpene in special glands and release it as a defensive secretion when they are attacked by their enemies, such as ants.

However, surprising results emerged after comprehensive biochemical characterization of the enzyme. "After we had conducted an in vitro analysis of the protein, including measurements of product formation in the presence of different metal ions as co-factors, we were surprised to discover that only geranyl diphosphate (C10), a precursor for the defensive substance chrysomelidial, was produced after addition of cobalt and manganese ions. On the other hand, adding magnesium ions resulted in the formation of farnesyl diphosphate (C15), a potential precursor for juvenile hormones, which is 5 carbon atoms longer," says the scientist. All three metals were found in larval tissue, leading to the assumption that enzyme catalysis is directed by the different metal co-factors in the larvae, whichever is predominant in amount: Towards toxin or hormone – physiologically a major difference.

Sequence comparisons cannot replace a thorough

biochemical analysis

How the different metal ions modify the product range of the enzyme is still unclear. It is very likely that the varying atomic radii of the metal ions involved in the catalysis effect changes in the spatial structure of the enzyme, which prevent or allow the admission of a third C5 unit and hence result in the production of C10 or C15 molecules.

"Our experiments provide two important findings," says Wilhelm Boland, director at the Max Planck Institute. "First, the directing influence of metal ions on the product formation of isoprenyl diphosphate synthases is a novel "control element" in the regulation of the terpene metabolism which should be included in future experimental settings. And secondly: The diversity of terpenoid molecules cannot be attributed solely to the broad substrate specificity of some enzymes in the last steps of the metabolic pathway, but is in fact already inherent in early biosynthetic steps." Nature continues to provide interesting answers to the question how organisms manage to produce tens of thousands of different secondary metabolites. [JWK/AO]

More information: Sindy Frick, Raimund Nagel, Axel Schmidt, René R. Bodemann, Peter Rahfeld, Gerhard Pauls, Wolfgang Brandt Jonathan Gershenzon, Wilhelm Boland, Antje Burse: Metal ions control product specificity of isoprenyl diphosphate synthases in the insect terpenoid pathway. *Proceedings of the National Academy of Sciences*, Early Edition, February 25, 2013, DOI:10.1073/pnas.1221489110

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