Researchers study the formation of cardenolides in plants

September 18 2023

Both species produce high levels of cardenolides, although they belong to different plant families. Comparative analyses based on genomic, transcriptomic and metabolomic data helped to identify the enzymes involved in the biosynthesis of pregnenolone. Credit: Angela Overmeyer, Max Planck Institute for Chemical Ecology

Scientists at the Max Planck Institute for Chemical Ecology in Jena are investigating the previously largely unknown biosynthetic pathway that leads to the formation of cardenolides in plants.

In a study published in the journal *Nature Plants*, they present two enzymes from the CYP87A family as key enzymes that catalyze the formation of pregnenolone, the precursor for the biosynthesis of plant steroids, in two different plant families. The discovery of such enzymes should help to develop platforms for the cheap and sustainable production of high quality steroid compounds for medical use.

Plants produce an impressive array of metabolites, including many medically valuable steroids. Well-known examples of this class of substances obtained from plants are cardenolides. As early as 1785, the British physician William Withering (1741–1799) published a book on the red foxglove and its use in medicine (An account of the foxglove, and some of its medical uses: with practical remarks on dropsy, and other diseases. Birmingham 1785).

He had found out in experiments that taking extracts of the plant increased the flow of urine in sick people, thus treating water retention in the body. However, he did not know that the active ingredients in foxglove leaves had a direct effect on the heart.

Since the second half of the 19th century, cardenolides, cardiac glycosides from plants, have been used to treat of heart failure or arrhythmia because of their effect on the heart muscle.

"In addition to their effect on the contractility of the heart, cardenolides have been used with great success in recent years for the treatment of various cancers. However, the corresponding plant biosynthetic pathways have remained largely unknown despite the success of these steroid molecules in human medicine. Our goal was therefore to understand how plants synthesize these highly complex molecules from predicted but simple precursors," explains first author Maritta Kunert.

In addition to foxglove Digitalis purpurea, the research team also studied another plant species, the rubber tree Calotropis procera. Although these two plants belong to different plant families, they both produce large amounts of cardenolides.

Since the species studied are not model plants whose genomes have been sequenced and for which many gene functions are known, the project was initially something of a "black box" for the researchers, as they had no existing data sets or standard methods to fall back on.

The starting point for the study was earlier work in a related species of foxglove, which suggested that the biosynthesis occurred via the molecule pregnenolone, sometimes referred to as the "mother of all steroid hormones" because all major steroid hormones such as testosterone, progesterone and estrogen in humans can be traced back to the precursor pregnenolone.

"We identified the candidate genes involved in cardenolide biosynthesis by comparative analysis of the two plant species. The structures of the cardenolides in these plants have both overlapping and divergent profiles. Therefore, comparing information about the plants' genomes, in particular which genes are expressed in these two plants in relation to the formation of metabolites, was very helpful in identifying the enzymes involved in the formation of pregnenolone," says study leader Prashant Sonawane, who heads the project group "Steroidal Specialized Metabolism in Plants" in the Department of Natural Product Biosynthesis.

In addition, the scientists did not even know where the metabolites of interest were accumulated in the different parts of plants. "The tissuespecific localization of the cardenolides was crucial for using the genetic data sets in a way that allowed the selection of 13 candidate genes. Comparing these datasets across different plants helped us to reduce the number of candidate genes for further characterization," explains Prashant Sonawane.

Finally, two enzymes of the cytochrome P450 family 87A were identified that catalyze the conversion of both cholesterol and phytosterols into pregnenolone in foxglove and Calotropis procera. This was the first step in the cardenolide biosynthetic pathway in these two only distantly related plants. Importantly, this is the first enzymatic function reported for this subfamily of cytochrome P450.

The scientists tested their findings by modifying plants of the model system Arabidopsis thaliana to produce more CYP87A enzymes. The genetically modified Arabidopsis plants accumulated unusually high levels of pregnenolone.

Further evidence for the involvement of CYP87A enzymes in the formation of pregnenolone came from genetically modified foxglove plants that lacked CYP87A enzymes in their leaves. In these plants, the formation of pregnenolone and cardenolides was greatly reduced. The authors established the first stable transformation system to modify foxglove plants for the study of specialized metabolites.

The research team is far from satisfied with deciphering the first enzymatic step of cardenolide biosynthesis. "We are already working on the downstream steps for the formation of cardenolides in different plant species. This biosynthetic pathway is long and highly complex. With the ability to apply the latest sequencing, bioinformatics and metabolomics methods across multiple plant species, we hope to solve this puzzle soon," says Prashant Sonawane.

Plants produce many pharmaceutical compounds. The extraction of these natural products is still very complex and often not very sustainable. The Department of Natural Product Biosynthesis at the Max Planck Institute for Chemical Ecology, led by Sarah O'Connor, aims to elucidate the biosynthetic pathways of important phytochemicals with medical relevance.

"The discovery of enzymes such as CYP87A can help develop biological platforms for the sustainable production of high-value plant compounds by using other plants for their biosynthesis," says Sarah O'Connor.

More information: Maritta Kunert et al, A promiscuous CYP87A enzyme activity initiates cardenolide biosynthesis in plants, *Nature Plants* (2023). DOI: 10.1038/s41477-023-01515-9

Provided by Max Planck Society

Citation: Researchers study the formation of cardenolides in plants (2023, September 18) retrieved 28 April 2024 from https://phys.org/news/2023-09-formation-cardenolides.html