Production of mustard oils: On the origin of an enzyme

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Plants of the mustard family, such as cabbage, produce glucosinolates that help to fend off herbivorous insects by reacting as part of “mustard oil bombs”. Inserted into the photo is a three-dimensional model of the enzyme IPMS, which is involved in the formation not of glucosinolates, but of the amino acid leucine. In the course of evolution the enzyme IPMS was converted to MAM: 120 amino acids disappeared - represented as pale-colored in the picture - and two mutations in the active site of the molecule occurred. Since then, MAM’s role has been to synthesize precursors of glucosinolates. Credit: MPI for Chemical Ecology/A. Schneider; enzyme model based on Koon, PNAS 101, 2004

(PhysOrg.com) -- In the evolutionary arms race, small changes can be sufficient to gain a crucial advantage over the enemy. Scientists at the Max Planck Institute for Chemical Ecology found out recently that the ancestor of a gene involved in making chemical defenses in plants of the mustard family (Brassicaceae), such as rapeseed and cabbage, originally

had a completely different function, playing a part in the formation of leucine, an essential amino acid for humans. Only small changes in the structure of the enzyme enabled it to take over completely new tasks that could, as shown in this study, increase the survival advantage of the plants.

Plants are continually exposed to herbivore attack. To defend themselves, they have developed sophisticated chemical defense mechanisms. Plants of the mustard family, such as thale cress (Arabidopsis thaliana), produce glucosinolates (mustard oil glucosides) to protect themselves against herbivory. Scientists know many different kinds of these molecules; they have a similar structure, but different side chains. If insect larvae feed on mustard plants, glucosinolates are hydrolyzed to form toxic isothiocyanates. Chemists call this the “mustard oil bomb”.

Special enzymes are responsible for catalyzing the synthesis of different side chains of the various glucosinolates. Scientists at the Max Planck Institute for Chemical Ecology in Jena have now isolated one of these enzymes from Arabidopsis thaliana and discovered a surprising new insight. Jan-Willem de Kraker and Jonathan Gershenzon reported that the enzyme methylthioalkylmalate synthase (MAM), which catalyzes glucosinolate formation, strongly resembles another enzyme with a completely different function: The enzyme IPMS (isopropylmalate synthase) is involved in the synthesis of the amino acid leucine. However, the scientists found two major structural differences between IPMS and MAM: the last 120 amino acids are absent in MAM, and in the active site of the enzyme, where the substrates react, two amino acids had been exchanged.

IPMS encoding genes are present in eubacteria, archaebacteria, algae and higher plants, but not in animals. Therefore, humans must ingest leucine as an essential amino acid with our food. In the model plant

Arabidopsis thaliana IPMS consists of a chain of 631 amino acids. In actual enzymes, these amino acid chains, also called polypeptides, are not straight. Depending on the sequence of the respective amino acids, chains are folded into helices, sheets and other shapes necessary for the polypeptide to perform its biological function. Thus the function of IPMS is to bind 2-oxoisovalerate and acetyl-CoA and thereby produce leucine precursors. To make sure that enzyme mediated catalysis does not happen uncontrolled in the cell, many enzymes are regulated by a feedback mechanism. In IPMS this mechanism is located in the last 120 amino acids of the polypeptide chain. Here, the enzyme receives a signal from the cell as soon as enough leucine is available and so stops producing leucine precursors. “We found that the missing 120 amino acids not only inactivate the regulation of enzyme activities, but also change the architecture of MAM completely,” Jonathan Gershenzon says. The missing 120 amino acids cause a profound change in the active site: it expands and now is able to bind larger substrates, and can therefore produce completely new products. For this reason MAM synthesizes the precursors of glucosinolates, not leucine.

The Max Planck researchers came across IPMS when they were looking for genes involved in glucosinolate production. In the context of these studies they isolated and sequenced the IPMS gene. The scientists assume that after a duplication of the IPMS DNA sequence millions of years ago, the “twin DNA” lost the fragment encoding the sequence of the last 120 amino acids. In the course of evolution this probably happened during the origin of the mustard family. The loss of the 120 amino acids turned out to be very advantageous for the plants: it enabled them to produce glucosinolates as a defense against herbivores. During further evolution, individual mutations in the active site of the emerging MAM enzyme occurred that accelerated the synthesis of glucosinolates by better binding of the substrates.

The assumptions could be confirmed by de Kraker and Gershenzon in

extensive in vitro experiments. The way MAM emerged is probably typical for the way new phenotypes arise from the variety of genetic information encoded and stored in DNA. It is another example of how small changes can lead to the development of new weapons in the evolutionary arms race between plants and herbivores.

More information: Jan-Willem de Kraker and Jonathan Gershenzon, From Amino Acid to Glucosinolate Biosynthesis: Protein Sequence Changes in the Evolution of Methylthioalkylmalate Synthase in Arabidopsis. *The Plant Cell* Vol. 23: 38-53; doi:10.1105/tpc.110.079269

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