

Scientists unlock mystery of how the 22nd amino acid is produced

March 30 2011

The most recently discovered amino acid, pyrrolysine, is produced by a series of just three chemical reactions with a single precursor – the amino acid lysine, according to new research.

Scientists at Ohio State University used mass spectrometry and a series of experiments to discover how cells make the amino acid, a process that until now had been unknown.

They confirmed that pyrrolysine is made from enzymatic reactions with two lysine molecules – a surprising finding, given that some portions of its structure suggested to researchers that it might have more complex origins.

The research is published in the March 31 issue of the journal *Nature*.

Pyrrolysine is rare and so far is known to exist in about a dozen organisms. But its discovery in 2002 as a genetically encoded amino acid in methane-producing microbes raised new questions about the evolution of the genetic code. Pyrrolysine is among 22 amino acids that are used to create proteins from the information stored in genes. Proteins are essential to all life and perform most of the work inside cells.

This information about how it is produced – its biosynthetic pathway – offers a more complete understanding of how amino acids are made. And because of its rarity, this molecule is emerging as a handy tool for manipulating proteins in biomedical research. With its production

mechanism identified, scientists can use that information to devise ways to mass-produce similar or identical synthetic molecules for a variety of research purposes.

The Ohio State scientists had a genuine "ah-ha" moment over the course of the study. As part of their experimentation, they combined lysine with one other amino acid and some enzymes and expected this to produce what is called an intermediate – essentially, a piece of an amino acid that is generated in the biosynthesis process.

They had labeled the lysine so it would appear heavier than normal when observed using mass spectrometry. But one signal produced by the instrumentation had a much different mass than could be attributed to the intermediate.

"We weren't seeing this weird molecule made from two different amino acids that we were expecting. We were seeing the regular pyrrolysine molecule and all of it was coming from lysine. Every bit of it," said Joseph Krzycki, professor of microbiology at Ohio State and senior author of the study. "That was the only way we saw pyrrolysine, and all of it was labeled with lysine. That's the basic observation here. And it's a real surprise."

The finding that lysine was the only precursor was a surprise because the production process ended up being so simple – even though arriving at it was not a simple task, partly because some of the [chemical reactions](#) had never been observed before.

"What amazes me about the entire chemical pathway is that you need only three enzymes and two molecules of the same thing that together make one complete molecule that looks completely different from what you started with," said Marsha Gaston, first author of the paper and a doctoral student in microbiology. "You have one portion that looks

exactly like the precursor, but then you have another portion that enzymes are able to re-arrange in a way that is completely unique and never seen before."

Mass spectrometry, an analytic technique that enables precision in determining the mass of particles, ended up being critical to the discoveries, Krzycki noted. Liwen Zhang and Kari Green-Church of Ohio State's Campus Chemical Instrument Center/Mass Spectrometry and Proteomics Facility are additional co-authors of the study.

Krzycki led one of the two teams of Ohio State researchers that discovered pyrrolysine in 2002. The teams have since synthesized the amino acid and shown how bacteria incorporate it into proteins.

"That left some big questions unanswered: How do you make pyrrolysine? Where does it come from? What metabolic pathways does it come off of? Because it's got to be generated within the cell that uses it," Krzycki said.

The chemical shape of pyrrolysine offered some clues. Its carbon skeleton resembles that of lysine. But it also has an unusual ring on one end, and a methyl group attached to it, which for researchers raised questions about its origin.

The researchers also knew from their previous work that three genes are required to generate the instructions for the assembly of proteins that contain pyrrolysine – *pylB*, *pylC* and *pylD*. So the enzymes produced by those three genes had to have a role in creation of the amino acid. Finally, previous attempts by other researchers to define its biosynthesis suggested that another amino acid, D-ornithine, was involved in pyrrolysine's production.

So Krzycki and his colleagues set out to test that theory. Conducting all

of their experiments in a strain of *E. coli* bacteria, commonly used to test biological functions, they combined lysine and D-ornithine molecules.

They found that this didn't make pyrrolysine, but rather a molecule like pyrrolysine that was missing a key part; however, this molecule turned out not to be converted to pyrrolysine. This molecule also was formed without the involvement of *pylB* – a gene that could not be left out of the process that actually makes pyrrolysine.

With the mass spectrometry instead identifying lysine as the only precursor to pyrrolysine, the researchers then used genetics, [mass spectrometry](#) of intermediates and deduction to determine the order of enzymatic reactions that converted two lysine molecules into the pyrrolysine amino acid.

They determined that the sequence of events matched the alphabetical order of the three involved enzymes: *PylB* uses lysine to make a D-ornithine-like intermediate, *PylC* joins the two lysine molecules together, and that feeds a reaction involving *PylD* that results in the formation of pyrrolysine. The reactions showed how the ring on pyrrolysine's end, its major identifying characteristic, is formed.

"If you splay out the pyrrolysine molecule, you can recognize that in fact it looks a lot like lysine, except that to get to this ring, you have to make the second molecule one carbon unit shorter," Krzycki said. "The lysine goes through a type of enzymatic reaction called a mutase reaction, where the carbon skeleton is rearranged to make this shorter molecule, which is like D-ornithine, but with one extra carbon now hanging off the chain in a new place. That's what one of our pyrrolysine biosynthetic enzymes, *PylB*, is doing."

Krzycki noted that this finding will add fuel to discussions of how the genetic code evolved. For example, the co-evolutionary theory suggests

that amino acids arising from a common precursor have similar codon assignments. Codons are three-letter "words" identifying the bases that DNA uses to specify particular [amino acids](#) as building blocks of proteins. Normally, codons signal the start or end of a protein, or a particular amino acid used to construct it.

"For the scientists who are devoted to exploring how the genetic code evolved, our data provides new insights that can feed the various theories for how the code evolved; the co-evolutionary theory is just one such example," Krzycki said.

The finding that pyrrolysine derives entirely from lysine means that pyrrolysine is part of the aspartic acid family in bacteria and Archaea, a group of single-cell microorganisms that are similar to bacteria in size and shape, but with a different evolutionary history. The microbes known to contain pyrrolysine are in the Archaea domain, and are able to convert a common class of compounds – the methylamines – into methane gas.

Provided by The Ohio State University

Citation: Scientists unlock mystery of how the 22nd amino acid is produced (2011, March 30) retrieved 24 April 2024 from

<https://phys.org/news/2011-03-scientists-mystery-22nd-amino-acid.html>

This document is subject to copyright. Apart from any fair dealing for the purpose of private study or research, no part may be reproduced without the written permission. The content is provided for information purposes only.