

Press release

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On the path to tailored enzymes:

Enzymatic synthesis of pyrrolysine, the mysterious 22nd amino acid

With few exceptions, all known proteins are using only twenty amino acids. 25 years ago scientists discovered a 21st amino acid, selenocysteine and ten years ago a 22nd, the pyrrolysine. However, how the cell produces the unusual building block remained a mystery. Now researchers at the Technische Universität München have elucidated the structure of an important enzyme in the production of pyrrolysine. The scientific journal "Angewandte Chemie" reports on their results in its "Early View" online section.

Proteins are key players in many vital processes in living organisms. They transport substances, catalyze chemical reactions, pump ions or recognize signaling molecules. The complexity and variety of proteins is tremendous, in the human body alone there are more than 100,000 different proteins at work. But almost all of them are made up of just twenty different amino acids. Only a few highly specialized proteins additionally contain selenocysteine, the very rare 21st amino acid discovered in 1986.

A big surprise was the discovery of a 22nd amino acid in methane-producing archaea of the family *Methanosarcinaceae* in 2002: pyrrolysine. It is genetically encoded in a similar manner as that of selenocysteine and the other twenty amino acids. The archaea use the unusual amino acid in proteins that they need for energy conversion. Pyrrolysine is located in the catalytic center of the proteins and is essential for their function. The energy generation process of the archaeobacteria would not work without pyrrolysine.

In March 2011, scientists at Ohio State University succeeded in deciphering parts of the manufacturing process of pyrrolysine. They proposed a reaction mechanism suggesting that the enzyme PylB catalyzes the first step of pyrrolysine biosynthesis by converting the amino acid lysine to the intermediate product methylnornithine. Scientists headed by Michael Groll, Professor of Biochemistry at the TUM-Department of Chemistry, could now elucidate the crystalline structure of PylB using X-ray structure analysis.

To their great surprise, they caught the enzyme literally "in the act": at the time of crystallization the reaction product, methylnornithine, had not left the enzyme. It adhered to a

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confined space, a kind of "reaction vessel", still in connection with the centers of the enzyme responsible for its creation. "That the product was still present in the enzyme, was something special and a great stroke of luck," says Felix Quitterer, a member of the scientific staff at the Department of Biochemistry and lead author of the publication. "We were not only able to directly detect the methylornithine, but also retroactively reconstruct how it is created from the source amino acid lysine."

This reaction was not only hitherto unknown, it is also very difficult to catalyze. It is a cluster of four iron and four sulfur atoms in the active site of the enzyme that is the key to the conversion. "This is a really unusual enzymatic reaction. Up to now no chemist in the laboratory is able to synthesize methylornithine in a one-step reaction starting from lysine," says Michael Groll.

The conversion of lysine to methylornithine is helping scientists to understand how archaeobacteria can modify an existing system to enable the formation of a tailored amino acid that, when installed in the appropriate protein, catalyzes a very specific reaction. Researchers can use this knowledge to create artificial amino acids for "custom tailored" enzymes with special properties that could, for example, find applications in industrial biotechnology and medicine.

There is, however, a more fundamental reason for the great interest in the synthesis of the 22nd amino acid: Scientists are hoping to find new clues to the evolutionary development of the amino acid canon. Why does the vast complexity of proteins in living organisms descend from only a few natural amino acids, even though the genetic code would be able to encode many more? An answer to this fundamental question on the minimum requirements for life has thus far eluded scientists. Selenocysteine and pyrrolysine are exotic exceptions. But knowledge about their development from the standard amino acids helps to come a little closer to the answer.

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Original publication:

Crystal structure of methylornithine synthase (PylB): Insights into the pyrrolysine biosynthesis. Felix Quitterer, Anja List, Wolfgang Eisenreich, Adelbert Bacher and Michael Groll, *Angewandte Chemie*, Early View, 16. Nov. 2011 – DOI: 10.1002/ange.201106765 Link: <http://onlinelibrary.wiley.com/doi/10.1002/anie.201106765/abstract>

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