BIOSYNTHESIS

Alkyne amino acid biosynthesis

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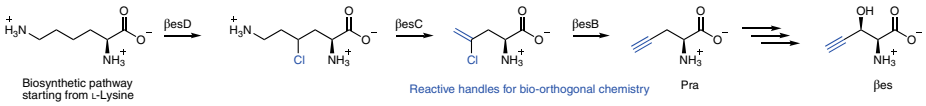

Equipping proteins with non-standard amino acids for bio-orthogonal chemistry is a powerful technology for their modification and investigation in a cellular environment. Usually, the non-standard amino acids are fed to the organism, which is cost-expensive. Thus, the identification of biosynthetic pathways for the in situ production of these unusual amino acids directly from glucose is from particular interest.

Now, Michelle C. Y. Chang and colleagues at the University of California, Berkeley, have discovered a biosynthetic pathway for the production of halo, terminal-alkyne and terminal-alkene amino acids that can be incorporated into proteins for subsequent bio-orthogonal reactions such as the Cu(I)-catalysed azide–alkyne cycloaddition (CuAAC), or olefin methatesis.

The bacterium *Streptomyces cattleya* was known to produce β-ethynylserine (βes) — an amino acid with a terminal alkyne — but the genes involved in the biosynthetic pathway remained unclear. Chang and colleagues identified the responsible βes gene cluster by comparing genomes from bacteria that produce and do not produce alkyne-containing amino acids and by subsequent analysis of the predicted function of the conspicuous genes.

Gene knockout experiments confirmed the role of the gene cluster in βes production. Moreover, by comparative metabolomics experiments, putative intermediates and precursors of this biosynthetic pathway could be identified. The involved enzymes were isolated, allowing their investigation in more detail. In addition, the full βes pathway could be reconstituted in vitro.

Interestingly, the discovered pathway includes several non-standard L-amino acids, such as 4-chlorolysine, allylglycine and propargylglycine (Pra). The amino acids could also be produced in *E. coli*. Moreover, an engineered aminoacyl-tRNA synthase (PraRS) enabled the proteome-wide incorporation of the in situ produced alkyne-containing amino acid Pra in *E. coli*, which was further confirmed by a click reaction with an azide-containing dye.

Overall, this work provides a powerful and cheap method for the in situ production of non-standard amino acids to modify the space of chemical functional groups in proteins and proteomes, offering a manifold of opportunities in bio-orthogonal chemistry and xenobiology.

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