Investigation of the Biosynthetic Process of Indigoidine | Biological Engineering

07/19/2019

Yi Chen

Master's Defense
Department of Biological Engineering

Monday, July 29
10:00 AM

Advisor - Jixun Zhan
Jixun.Zhan@usu.edu

Full Abstract

Indigoidine (5,5#-diamino-4,4#-dihydroxy-3,3#-diazadiaphenoquinone-(2,2#)) is a non-ribosomal peptide synthetases (NRPS)-associated natural product. It has been proved with antioxidant and antimicrobial activity, and can be used as a reporter system for gene expression. It features a unique dark blue color similar to industrial dye indigo, thus named as indigoidine. As a blue pigment, it has the potential to be produced in large scale for industrial use. Though different strains have the ability to produce indigoidine, some hosts have the genes silenced, while others have very limited production of indigoidine. Thus, heterologous expression of the responsible synthetase gene and a good understanding of the biosynthetic mechanism will significantly benefit its large-scale production and industrial applications.

Previously, we cloned a putative indigoidine synthetase gene (Sc-indC) from Streptomyces chromofuscus ATCC 49982. Heterologous expression of the Sc-indC in E. coli achieved indigoidine production of 20 g/L under optimized conditions. Exploring the mechanisms of indigoidine synthesis will potentially open new doors to further improve the production of the industrially-important natural dye.

As a non-ribosomal peptide synthase (NRPS), the indigoidine synthetases were predicted to have four functional domains: thiolation (T), thioesterase (TE), and a special oxidation (Ox) integrated with the adenylation domain (A). It was proposed that the A domain activated the substrate L-glutamine, and bound it to the T domain, where the TE domain catalyzed the cyclization to produce the intermediate Pyro-Gln. The intermediate compound was then released from T domain as a free molecule, and subsequently oxidized by Ox domain, resulting 5-amino-3H-pyridine-2,6-one, which was finally dimerized to produce indigoidine. However, the proposed biosynthetic mechanism has not been justified and the sequence of the oxidation and cyclization still remains unknown.

Though in vivo expression showed Sc-IndC of higher activity, we found it had relatively lower in vitro activity compared to blue-pigment indigoidine synthetase (BpsA), which had the same functional domains as Sc-IndC. So, my study was dedicated on the biosynthetic mechanism of BpsA and Sc-IndC, of which the corresponding genes bpsA and Sc-indC were derived from two different bacteria.

Firstly, I cloned and heterologously expressed bpsA, Sc-indC, and the domain gene fragments of different combination in E. coli BAP1, and then purified the recombinant proteins. I chemically synthesized the analogue of the biosynthetic intermediates, L-glutamine-SNAC, which mimicked the thiolated glutamine in the biosynthetic process to work as an intermediate. Finally, I optimized the in vitro enzyme assay conditions to test
the enzymatic activity of the purified whole enzymes and domains on the substrate L-glutamine, the intermediates L-glutamine-SNAC and 3-aminopiperidine-2,6-dione (Pyro-Gln). The products were analyzed by LC-MS to reveal the biosynthetic process of indigoidine by BpsA and Sc-IndC.