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Crystallographic analysis of enzymes involved in the biosynthesis of natural products possessing complicated chemical structures

Takeo TOMITA[1,2] Masaya Kobayashi[1] and Tomohisa KUZUYAMA[1,2,*]
[1] Biotechnology Research Center, The Univ. of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo, 113-8657, Japan
[2]CRIIM, The Univ. of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan

1 Introduction

Carquinostatin A (CQS) is a carbazole alkaloid isolated from an actinomycete *Streptomyces exfoliates*. CQS exhibits antioxidant activity, and is thought to act as neuroprotective agent in cerebral ischemia. Therefore, it is expected to be a lead compound for inhibition of neurodegenerative diseases.

Recently, we identified gene cluster responsible for CQS biosynthesis through heterologous production and gene deletion [1]. Biochemical characterization of seven CQS biosynthetic gene products (CqsB1-7) established the total biosynthetic pathway of CQS. We found that CqsB2 cyclizes an unstable biosynthetic intermediate with acyl side chain to create carbazole intermediate, precarquinostatin (Fig. 1).

In this project, we conducted crystallographic analysis of CqsB2 to elucidate the mechanism of unprecedented enzymatic reaction.

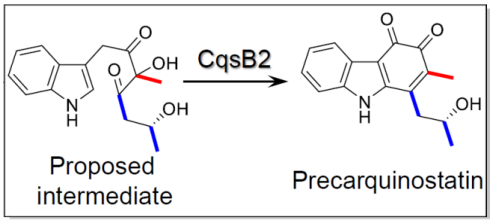

Fig. 1: The reaction catalyzed by CqsB2

2 Experiment

Protein expression and purification

E. coli BL21-CodonPlus (DE3)-RIL cell possessing pHIS8-CqsB2, which is a plasmid for expression of CqsB2 fused with histidine tag at the N-terminal, was grown in 2 x YT broth in the presence of kanamycin (50 μg/ml) and chloramphenicol (30 μg/ml) at 303 K. The gene expression was induced by adding 0.1 mM IPTG and the culture was continued for additional 12-14 h. The samples were purified with Ni^{2+}-resin column chromatography, and Superdex 200 gel filtration column chromatography. The homogeneity over 95 % of the purified CLDS were verified by SDS-PAGE.

E. coli B834 (DE3) was transformed with pHIS8-CqsB2 to express SeMet-CqsB2 protein. The transformant was grown in SeMet Core medium and the gene expression was induced by adding 0.1 mM IPTG. The protein was purified with the same method with that of native CqsB2 protein.

Crystallization

Crystallization conditions were screened by the hanging-drop vapor-diffusion method using Crystal Screen (Hampton Research) and the Wizard crystallization screen series (Emerald Bio). The screenings were set up using 2 μl drops consisting of 1 μl reservoir solution and 1 μl 10 mg ml^{-1} CqsB2 solution with and without 2 mM intermediate precarquinostatin.

3 Results and Discussion

We determined crystal structure of CqsB2 by the single-wavelength anomalous diffraction (SAD) method with a SeMet-substituted protein [1]. CqsB2 consists of N-terminal arm and C-terminal core domain, which adopts the similar structure with TcmN aromatase/cyclase, and forms a homo-dimeric structure (Fig. 2). Precarquinostatin binds at the active site (Fig. 3A). The docking model bound with the substrate was created based on the crystal structure (Fig. 3B). The CqsB2 structure and structure-based mutagenesis study provided mechanistic insights into the reaction of CqsB2.

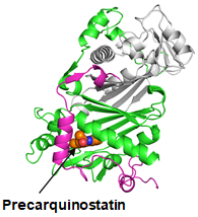

Fig. 2: Crystal structure of CqsB2

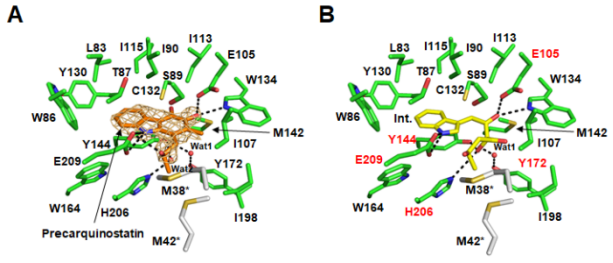

Fig. 3: Active site structure of CqsB2. (A) Active site bound with the CqsB2 product, precarquinostatin. (B) Docking model bound with the CqsB2 substrate.

References

[1] M. Kobayashi *et al*., *Angew. Chem. Int. Ed. Engle*. **58**, 13349 (2019).

* utkuz@mail.ecc.u-tokyo.ac.jp