BL-1A/2016G507

X-Ray Crystal Structure Analysis of Plant Type III Polyketide Synthase

Chin Piow WONG[1] and Hiroyuki MORITA[1,*]
[1]Institute of Natural Medicine, University of Toyama, Toyama 930-0194, Japan

1 Introduction

Type III polyketide synthases (PKSs) perform C-C bond forming reactions by iterative Claisen-type condensation of CoA thioesters and cyclization of the poly-*β*-keto intermediates, to produce pharmaceutically and biologically important aromatic polyketides, such as chalcone, stilbene, and curcumin. Tetraketide synthase PoOLS (*Primula obconca* olivetol synthase) is a plant derived type III PKS involved in the biosynthesis of primin. PoOLS is proposed to catalyze the condensations of one hexanoyl-CoA and three malonyl-CoA to generate a linear pentyltetraketide-CoA, which is cyclized by another enzyme into olivetolic acid and further modified into the final product primin (Figure 1). Apart from its native substrate, PoOLS is able to accept CoA substrates with sidechain up to 11 carbons as starter unit. In effort to understand substrate and product specificities, we proceed to determine the X-ray crystal structure of PoOLS.

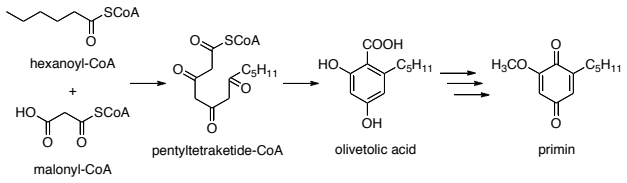

Fig. 1: The proposed biosynthetic pathway of primin.

2 Materials and Methods

Crystallization – Diffraction-quality crystals of PoOLS were obtained after few days of incubation at 4 °C, in 100 mM Tris-HCl (pH 8.8) containing 20% PEG2000, with 22 mg/mL of purified PoOLS solution, by using sitting-drop vapor-diffusion method.

Data collection – The crystals were transferred into the soaking solution with 5% (v/v) glycerol for 10 sec for cryoprotection and then flash cooled at -173°C in a nitrogen-gas stream. The X-ray diffractions of crystals were collected at BL1A, processed and scaled with *XDS*. The structure was solved by the molecular replacement method with *Phaser-MR (simple one-component interface)* using alkylquinolone synthase (AQS, PDB accession code: 5wx4) as search model. The structure was modified manually with *Coot* and refined with *PHENIX*.

3 Results and Discussion

The crystal structure of PoOLS was solved at 1.8 Å. The final *R*-value was 20.6% (R_{free} = 26.6%). PoOLS formed a symmetric dimer and showed the typical type III pilyketide synthase fold. Significant difference between the overall structures of PoOLS and the other type III PKSs were not observed (Figure 2). Homology search using the Dali Program [1] indicated that PoOLS is most homologically related to OsPKS, a type III PKS identified from *Oryza sativa*, with Cα RMSD at 0.9 Å. PoOLS catalytic cavity is tunnel-shaped, resembling the catalytic cavity of PKS18, a mycobacterial type III PKS [2]. The obtained result suggests that PoOLS favors C_6-C_{12} aliphatic-CoAs as a starter substrate to produce linear C_5-C_{11} fatty acyltetraketide-CoAs. However, further structure-based site-directed mutagenesis studies of PoOLS will provide insight into the catalytic versatility of the type III PKSs.

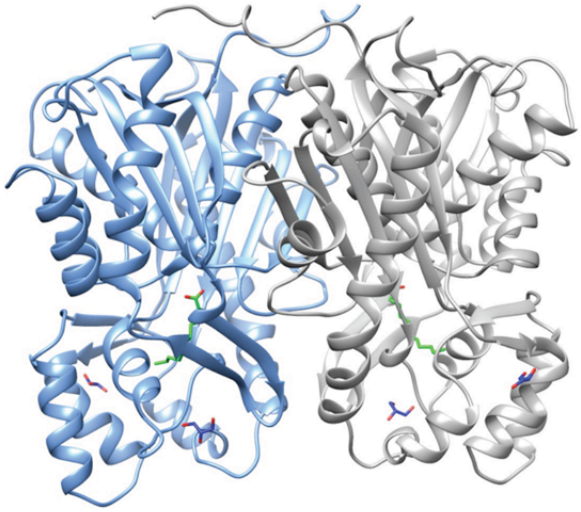

Fig. 2: Structure of the PoOLS. Lauric acid and glycerol are colored green and blue sticks, respectively.

Acknowledgement

We sincerely thank the PF staffs for their help with our data collections.

References

[1] L. Holm, C. Sander, *Trends Biochem. Sci.* **20**, 478-480 (1995).
[2] R. Sankaranarayanan, P. Saxena, U.B. Marathe, R.S. Gokhale, V.M. Shanmugam, R. Rukmini, *Nat. Struct. Mol. Biol.* **11**, 894-900 (2004)

* hmorita@inm.u-toyama.ac.jp