BL-17A, AR-NE3A, AR-NW12A/2015G007

Crystal structure analysis of plant polyketide cyclcase OAC from *Cannabis sativa*

Takashi Matsui[1], Xinmei Yang[1], Xiaoxi Zhou[1], Futoshi Taura[2], Hiroshi Noguchi[3], Ikuro Abe[4] and Hiroyuki Morita[1,*]

[1]Institute of Natural Medicine, University of Toyama, Toyama, 930-194, Japan

[2]Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, 930-0194, Japan

[3]School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, 422-8526, Japan

[4]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033, Japan

1 Introduction

Polyketides are a structurally diverse family of natural products. In the biosynthesis of plant polyketides, the construction of the ring formation is a key step in diversifying the polyketide structures. Olivetolic acid cyclase (OAC) derived from *Cannabis sativa* L., involved in cannabinoid biosynthesis, is the only known plant polyketide cyclase. OAC catalyzes the C2-C7 intramolecular aldol cyclization of linear pentyl tetra-β-ketide CoA to generate olivetolic acid (OA). To clarify the intimate catalytic mechanism of OAC, structures of several OAC mutants were determined.

2 Experiment

Crystallization – 20 mg/ml OAC I7F, Y27W, V59M, Y72F an H78S mutants were crystallized with sitting-drop vapor diffusion method, respectively. 1 μl of protein and 1 μl of reservoir solution were mixed, and equilibrated against 50 μl of reservoir solution at 5°C. Diffraction-quality mutants crystals were finally obtained after a few days incubation.

Data collection and structure determination – Crystals were transferred into the reservoir solution containing 10%(v/v) glycerol as a cryoprotectant, and were flash-frozen in a nitrogen stream. The X-ray diffraction data of mutant crystals were collected at beamline BL-17A, NE3A, and NW-12A under cryogenic condition at -173°C, respectively. The diffreaction data was processed and scaled usnig *XDS*. The structures of OAC mutants were solved by the molecular replacement method with *Molrep* or *Phaser* using OAC apo as the search model. The structures of OAC apo and OAC-OA complex modified mannually with *Coot* and refined with *phenix.refine*. The coordinates and structure factors have been deposited under accession number 5B0B for the I7F, 5B0D for the Y27W, 5B0E for the V59M, 5B0F for the Y72F and 5B0G for the H78S mutant, respectively.

3 Results and Discussion

X-ray structures of OAC I7F, Y27W, V59M, Y72F, and H78S were refined at 2.18, 1.80, 1.60, 1.60, and 1.40 Å resolutions, respectively. The final *R*-values of I7F, Y27W, V59M, Y72F, and H78S mutant structures were 20.1%, 19.6%, 21.2%, 17.5%, and 17.4% (R_{free} = 25.4%, 24.5%, 23.5%, 22.3% and 20.0%), respectively. The structural comparisons couplied with enzymatic reaction revealed that the Y72F and H78S, which lost the catalytic activity, substitutions only disrupt the hydrogen bond bwetween Tyr72 and His78 at the active site with no significant conformational change. Furthermore, The I7F, Y27W, and V59M mutants, which located on the pentyl-binding pocket and showed 15%, 63% and 35% decreases in OA-forming activity, exhibited estimated total cavity volumes of 247, 204, and 222 Å^3, which are slightly less than the 270 Å^3 volume of the wild type. These observations strongly favor the possible catalytic roles of Tyr72 and His78 over their roles in substrate binding affinity, and the crucial roles of the pentyl-binding pocket in the binding affinites of the pentyl moiety of the substrate, respectively.

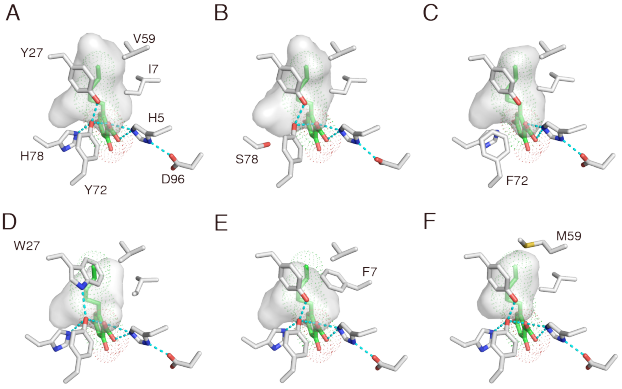

Fig. 1: The active-site architectures of wild-type OAC and the OAC mutant enzymes. The active-site architectures of (A) OAC and the OAC mutant enzymes (B) H78S, (C) Y72F, (D) Y27W, (D) I7F, and (H) V59M. The OA molecule bound in the wild-type OAC structure is superimposed on the active-site cavity of the mutant enzymes, and depicted by a green stick model. The van der Waals force of OA and the hydrogen bonds are depicted by dotted surfaces and light blue dashed lines, respectively.

References

[1] X. Yang *et al*., *Acta Cryst*. **F71**, 1470-1474 (2015).
[2] X. Yang *et al*, *FEBS J*. **283**, 1088-1106 (2016).

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