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

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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, OAC structure was determined.

2 Experiment

Crystallization – 17 mg/ml selenomethionine-labeled OAC with or without 50 mM olivetolic acid were crystallized with sitting-drop vapor diffusion method. 1 μl of OAC and 1 μl of reservoir solution were mixed, and equilibrated against 50 μl of reservoir solution at 5°C. Diffraction-quality apo crystals were finally obtained in 0.1 M Tris-HCl pH 8.8, 25%(w/v) PEG6000, 0.1 M sodium malonate after a few days incubation. OAC with olivetolic acid was co-crystallized 0.1 M Tris-HCl pH 8.8, 25%(w/v) PEG6000, 50 mM olivetolic acid and 5%(v/v) methanol 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. Single-wavelength anomalous diffraction (SAD) data of OAC apo crystal and diffraction data of OAC-OA crystal were collected at beamline NW-12A and NE-3A under cryogenic condition at -173°C, respectively. Wavelength of 0.97908 Å at NW-12A was used for data collection on the basis of the fluorescence spectrum of the Se *K* absorption egde. The diffreaction data was processed and scaled usnig *XDS*. Se sites were determined and refined, and the initial phase of OAC was calculated with *AutoSol*. The structure was rebuilt using *AutoBuild*. The initial phase of OAC-OA complex was calculated by the molecular replacement method with *Molrep* 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 5B08 for the OAC apo and 5B09 for the OAC-OA complex.

3 Results and Discussion

X-ray structures of OAC apo and OAC-OA complex were refined at 1.32 Å and 1.70 Å resolutions, respectively. The final *R*-values of apo and complex structures were 19.7% and 19.7% (R_{free} = 21.5% and 22.3%), respectively. The asymmetric unit of the binary complex crystal contains a monomer, which forms a biologically active symmetric dimer with a crystallographic two-fold axis. A comparison of the overall structure of OAC apo and the binary complex revealed that the structure of OAC-OA binary complex is nearly identical to the apo structure. The monomer of the OAC-OA complex consists of a four-stranded antiparallel β-sheet and three α-helices as seen in plant dimeric α+β barrel (DABB) protein (Fig. 1A). The binary complex structure indicated that OAC possesses an active-site cavity in the interior of the α+β barrel in each monomer to accommodate the product (Fig. 1B).

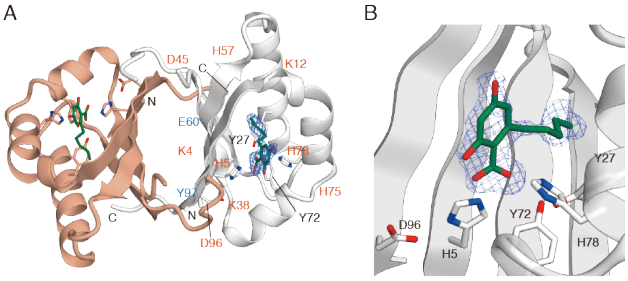

Fig. 1: Crystal structure of OAC-OA. (A) The homodimeric structure of OAC, formed by a crystallographic two-fold axis. (B) The active-site cavity of OAC-OA binary complex. Monomer in the asymmetric unit in the OAC-OA binary complex and its symmetric monomer are shown in white and wheat cartoon models, respectively. Electron density maps of OA are displayed with blue meshes ($F_o - F_c > 2.0\sigma$).

References

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