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X-Ray Crystal Structure Analysis of Type III Polyketide Synthase

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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. Octaketide synthase (OKS) from *Aloe arborescens* is a plant-specific type III polyketide synthase (PKS) that catalyzes condensations of eight molecules of malonyl-CoA to produce the aromatic octaketides SEK4 and SEK4b. We reported that structure-based OKS N222G single mutant efficiently produces an unnatural aromatic dekaketide SEK15 by condensations of ten molecules of malonyl-CoA. X-ray crystal structure of the OKS N222G mutant was also obtained, and demonstrated that the single amino acid substitution expanded the active-site cavity to accommodate ten malonyl units [1]. On the other hand, a structure-based active-site F66L/N222G double mutation of OKS results in a formation of an unnatural aromatic dodecaketide TW95a, by sequential 12 malonyl-CoA condensations [2]. The dodecaketide has been previously reported as a product of genetically engineered type II PKSs, and now represents the longest polyketide produced by the structurally simple type III PKS. To elucidate the structure function relationship of the OKS F66L/N222G double mutant, we determined X-ray crystal structure of the F66L/N222G mutant at PF.

2 Materials and Methods

Crystallization – 20 mg/mL F66L/N222G mutant was incubated with 5 mM CoA-SH at room temperature for 30 min, and then subjected to the crystallization with sitting-drop vapor diffusion method. 4 μl of the F66L/N222G protein and 2 μl of reservoir solution were mixed, and equilibrated against 500 μl of reservoir solution at 5°C. Diffraction-quality crystals were finally obtained in 0.1 M HEPES-NaOH (pH 7.2) containing 0.20-0.25 M ammonium sulfate and 22%(w/v) PEG3350 after a few days incubation.

Data collection – 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 diffractions of crystals were collected at BL5A and BL17A, processed and scaled with *XDS*. The structure was solved by the molecular replacement method with *Molrep* in the *CCP4* suite using OKS wild-type structure (Unpublished data) as the search model. The structure was modified manually with *Coot* and refined with *PHENIX*.

3 Results and Discussion

A X-ray structure of the OKS F66L/N222G mutant was refined at 2.49 Å resolution. The final *R*-value was 18.7% (R_{free} = 23.7%). Asymmetric unit contains two monomers, which form the biologically active symmetric dimer (Figure 1). The electron density map of the crystal indicated the presence of CoA-SH. The structure of the F66L/N222G double mutant suggested that the large-to-small F66L/N222G double mutations open a gate to a novel hidden pocket behind both of the active site of the OKS wild-type and its N222G single mutant, thereby expanding the putative polyketide chain-elongation tunnel, which leads to the formation of the longest dodecaketide TW93a. The finding would provide insight into further engineering of the type III PKSs, and further structure determination of the mutant complexed with the enzyme reaction intermediate are now in progress.

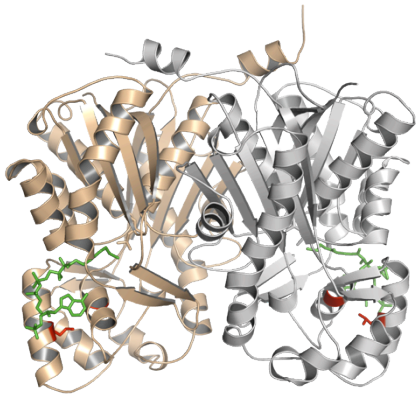

Fig. 1: Structure of the OKS F66L/N222G mutant. L66/G222 and CoA-SH are colored red and green sticks, respectively.

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References

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