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

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1 Introduction

Type III polyketide synthases (PKSs) catalyze the iterative Claisen-type condensation of the CoA thioesters and cyclization of the poly-*β*-keto intermediates, to produce pharmaceutically and biologically important compounds. Phenylethylchromone precursor synthase (PECPS) from agarwood, *Aquilaria sinensis*, is a type III PKS that is thought to be involved in the biosynthesis of 2-(2-phenylethyl)chromones (PECs), which is important aromatic ingredient in perfumery, oriental medicines, incensing ceremony, and crafts production [1]. Thus, to unveil the intimate catalytic mechanism of PECPS would provide insight into not only the catalytic diversity of type III PKSs, but also engineering of the enzyme to generate PEC analogs. Hence, we solved the crystal structure of PECPS.

2 Experiment

Crystallization – Diffraction-quality crystals of PEPCS were obtained at 20 ℃, in 100 mM Tris-HCl (pH 8.5) containing 0.12 M KF, 4% butanediol, and 24% PEG8000 with 15 mg/mL of purified PECPS solution, by using sitting-drop vapor-diffusion method.

Data collection – The crystals were transferred into the soaking solution with 20% (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 OsPKS (PDB entry 4YJY) as model. The structure was modified manually with *Coot* and refined with *PHENIX*.

3 Results and Discussion

The crystal structure of PECPS was solved by X-ray crystallography at 1.95 Å resolution. The final *R*-value was 19.9% (R_{free} = 23.7%). PECPS adopts the typical homodimeric construct and αβαβα-fold architecture that commonly occurs in other type III polyketide synthases (Fig. 1). Analyses of the cavity size using the CASTp Program [2] indicated that the catalytic cavity of PECPS (247 Å^3) is significantly smaller than OsCUS (642 Å^3), a type III PKS that catalyzes the one-pot formation of bisdemethoxycurcumin from the condensation of two *p*-coumaroyl-CoA and one malonyl-CoA [3]. Further analyses of amino acid residues lining the catalytic cavity of PECPS in conjunction with activity-based site-directed mutagenesis might provide further insight into the catalytic mechanism of PECPS.

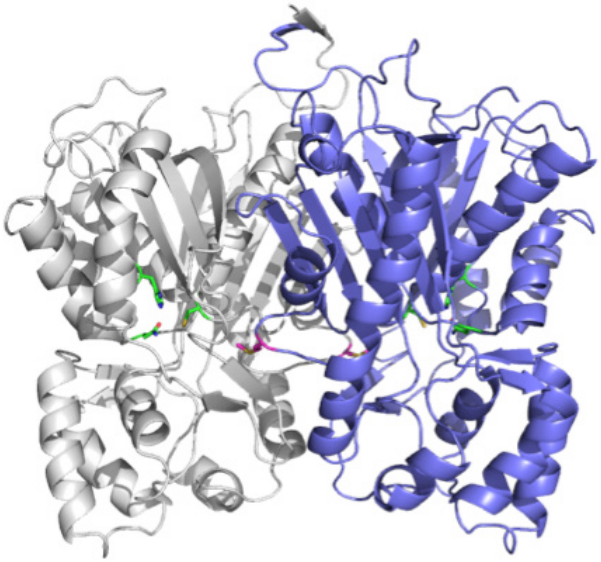

Fig. 1: Overall structure of the PECPS. The catalytic triad, Cys166, His308, and Asn338, is highlighted in green. Met139 protruding into adjoining monomer are highlighted in magenta.

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References

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