

X-ray crystal structure analysis of engineered *Cannabis sativa* tetraketide synthase that produces longer polyketide-CoA

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1 Introduction

Tetraketide synthase (TKS) from *Cannabis sativa* is a type III polyketide synthases (PKSs) that is involved in the biosynthesis of tetrahydrocannabinol. The enzyme catalyzes sequential condensation of three malonyl-CoA with hexanoyl-CoA and then, unlike most of other type III PKSs, produces a linear pentyl- β -tetraketide-CoA [1, 2]. The functional analysis has revealed that the enzyme accepts up to hexanoyl-CoA (C6) as the starter substrate. Currently, we found that substitution of Leu190 with Gly lining the active site cavity [2] of TKS led to dramatic changes of its substrate and product specificity in *in vitro* reaction, where the TKS Leu190G mutant enzyme has produced up to tridecanoyltetra- β -ketide-CoA by being conferred abilities to accept up to tetradecanoyl-CoA (C-14) with three malonyl-CoAs. The mutant enzyme may be available for the formation of cannabinoid analogues in the synthetic biology approach. In order to further clarify the detail mechanism, hence, we solved the crystal structure of the TKS Leu190G mutant enzyme.

2 Experiment

Crystallization – Diffraction-quality crystals of the mutant enzyme were obtained at 4 °C, in 200 mM ammonium acetate and 20% (w/v) PEG3350 with 20 mg/mL of purified TKS Leu190G mutant 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 TKS crystal structure (PDB entry 6GW3) as a template. The structure was modified manually with *Coot* and refined with *PHENIX*.

3 Results and Discussion

The crystal structure of the TKS Leu190G mutant enzyme was solved by X-ray crystallography at 2.10 Å resolution. The final *R*-value was 19.4% (*R*_{free} = 23.6%). The mutant enzyme adopts the typical homodimeric construct and $\alpha\beta\alpha\beta$ -fold architecture that commonly occurs in other type III polyketide synthases. Significant conformational changes were not observed in the overall structure between the wild type and mutant enzymes. In contrast, comparison of

the active site cavity of the wild type [3] and mutant enzymes revealed that the Leu190Gly substitution in TKS resulted in dramatically expanding the active site cavity to accept up to tetradecanoyl-CoA with three malonyl-CoAs, and thereby the mutant enzyme produced up to tridecanoyltetra- β -ketide-CoA as the products (Fig. 1).

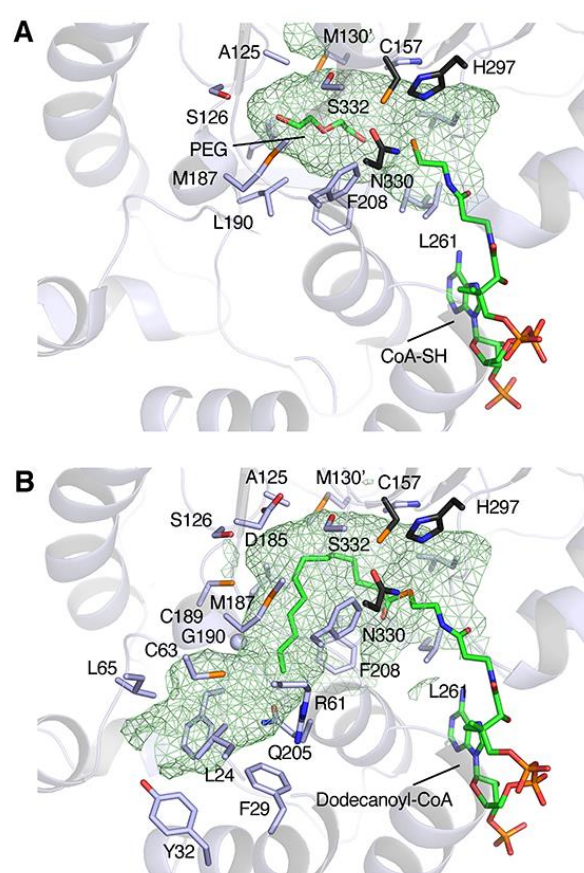


Fig. 1: Comparison of the active site cavity of (A) wild type (PDB code: 6GW3) and (B) TKS Leu190G mutant enzymes. The catalytic triad, Cys157, His297, and Asn330, is highlighted in black. The size and shape of the active site cavities are represented by green mesh.

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References

- [1] F. Taura *et al.*, *FEBS Lett.*, **2009**, 2061-2066.
- [2] S.J. Gange *et al.*, *Proc Natl Acad Sci USA*, **2012**, 109, 12811-12816.
- [3] L.J. Kearsey *et al.*, *FEBS J.*, **2020**, 287, 1511-1524.

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