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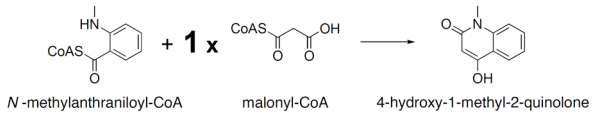
X-ray Crystal Structure Analysis of Quinolone-producing Novel Type III polyketide Synthase from *Citrus microcarpa*

Takahiro Mori[1], Yoshihiko Simokawa[1], Takashi Matsui[1], Hiroyuki Morita[2*] and Ikuro Abe[1*]
[1]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan
[2]Institute of Natural Medicine, University of Toyama, Toyama 930-0194, Japan

1 Introduction

Quinolone synthase (QNS) is a novel type III polyketide synthase (PKS) that produces diketide 4-hydroxy-1-methyl-2-quinolone as the single product by the one step condensation of *N*-methylanthraniloyl-CoA with malonyl-CoA (Scheme 1) [1]. Notably, *C. microcarpa* QNS exhibits quite unique substrate and product specificities. The enzyme did not accept 4-coumaroyl-CoA as a substrate and produced only triketide lactones from benzoyl-CoA and hexanoyl-CoA as a starter substrates. In contrast, the previously reported *Aegle marmelos* QNS did not produce the quinolone specifically, because it also generated the tetraketide 1,3-dihydroxy-*N*-methylacridone from *N*-methylanthraniloyl-CoA [2]. Furthermore, another major difference is that the quinolone- and acridone-forming *A. marmelos* QNS also accepted 4-coumaroyl-CoA to produce the diketide benzalacetone. To elucidate the structure function relationship of *C. microcarpa* QNS, we determined X-ray crystal structure of this enzyme at PF.



Scheme 1: Substrates and product of *C. microcarp* QNS.

2 Materials and Methods

Crystallization – 10 mg/mL *C. microcarpa* QNS was subjected to the crystallization with sitting-drop vapor diffusion method. 0.5 µl of the *C. microcarpa* QNS protein and equal volume of reservoir solution were mixed, and equilibrated against 50 µl of reservoir solution at 20°C. Diffraction quality crystals of *C. microcarpa* QNS were finally obtained in 50 mM Tris-HCl (pH 7.0), 18% (w/v) PEG3350, and 4% (v/v) 1-propanol, using the sitting-drop vapor diffusion method at 20°C.

Data collection – Crystals were transferred into the reservoir solution containing 18% (v/v) glycerol as a cryoprotectant, and were flash-frozen in a nitrogen stream. The X-ray diffractions of crystals were collected at BL17A, processed and scaled with the *HKL-2000*. The structure was solved by the molecular replacement method with *Molrep* in the *CCP4* suite using the *Medicago sativa* chalcone synthase (CHS) structure (Protein Data Bank code 1CGK) as the search model. The structure was modified manually with *Coot* and refined with *PHENIX*. The coordinates and structure factors have been deposited in the Protein Data Bank (Protein Data Bank code 3WD8).

3 Results and Discussion

A X-ray structure of *C. microcarpa* QNS was refined at 2.47 Å resolution [1]. The final *R*-value was 19.5% (R_{free} = 24.1%). Asymmetric unit contains four monomers A-D and monomers A/B and C/D form the biologically active symmetric dimers. Significant backbone changes were not observed between the monomers. The overall structure of *C. microcarpa* QNS (Fig. 1) is highly homologous to those of the structurally characterized plant type III PKSs (r.m.s.d. 0.7–1.3 Å). The catalytic triad consisting of Cys-164, His-303, and Asn-336 is buried deep within each monomer, at the intersection of a characteristic 16-Å-long CoA binding tunnel and a large cavity, in a location and an orientation very similar to those of the other plant type III PKSs. Ile-137 in *C. microcarpa* QNS, corresponding to Met-137 of *M. sativa* CHS, protrude into the other monomer and form part of the active site wall, as in the case of *M. sativa* CHS.

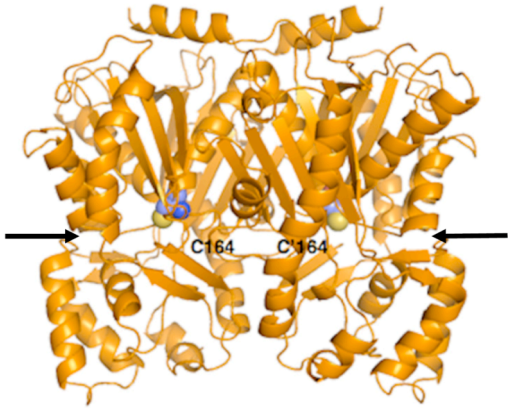


Fig. 1: Overall structure of *C. microcarpa* QNS. The catalytic Cys164 is represented by CPK model. Allows indicate the substrate entrance.

The crystal structure revealed that *C. microcarpa* QNS has an unusually wide active site entrance (Fig. 2). In the structure, the broadening of the active site entrance is caused by the F265L substitution and the deviation of the catalytic residue Cys-164 toward the outside of the active site cavity, as compared with the other type III PKSs. The wide active site entrance thus provides enough space to facilitate the access of the bulky *N*-methylanthraniloyl-CoA starter to the catalytic center of the enzymes.

In addition, the active site cavity of *C. microcarpa* QNS is quite small (290 Å^3) and losses the CHS's conserved coumaroyl-binding pocket, due to the unique substitutions of CHS's conserved Thr-132, Thr-194, and Thr-197 with Met, Met, and Tyr, respectively (Fig. 2). This is the reason why C. microcarpa QNS does not accept 4-coumaroyl-CoA as a substrate.

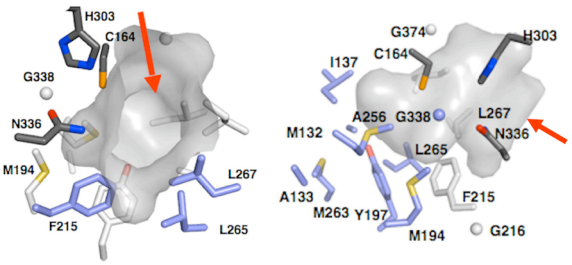


Fig. 2: Close-up view of active-site entrance (left) and structure (right) of *C. microcarpa* QNS. Allows indicate the substrate entrance in each structure.

Thus, the shape and the cavity volume of *C. microcarpa* QNS restrict the binding of the coumaroyl starter and the malonyl-CoA extender, suggesting that *C. microcarpa* QNS accepts the *N*-methylanthraniloyl-CoA starter through its wide active site entrance and catalyzes the condensation with malonyl-CoA. The chain elongation reaction is terminated at the diketide stage due to the steric contraction of the active site cavity, and this is followed by the N/C1 intramolecular lactamization of the Cys-bound linear diketide intermediate and concomitant thioester bond cleavage to produce the quinolone scaffold (Fig. 3).

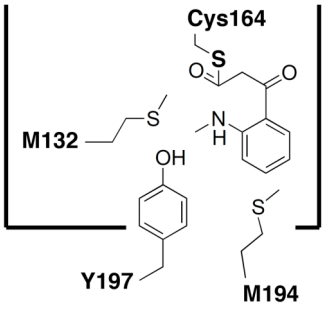


Fig. 3: Schematic representation of active structure of *C. microcarpa* QNS.

Acknowledgement

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References

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* hmorita@inm.u-toyama.ac.jp
* abei@mol.f.u-tokyo.ac.jp