Structural Elucidation of a Highly-Reducing Type II Polyketide Synthase

Polyketides are an important class of natural products produced by various microorganisms. Recently we found a novel class of type II polyketide synthase (PKS), a highly-reducing type II PKS (IgaPKS), by analyzing the ishigamide biosynthetic enzymes. To elucidate the molecular basis of IgaPKS, we solved the structures of its core catalytic heterodimer, ketosynthase–chain length factor (KS–CLF) complex, in three different states including hexanoyl unit- and acyl carrier protein (ACP)-bound forms. These structures and the results of site-directed mutagenesis provided important insights into ACP- and substrate-selectivity of the KS–CLF complex.

Polyketides are an important class of secondary metabolites showing useful biological activities, such as anticancer and antibacterial activities. They are biosynthesized by polyketide synthases (PKSs), which are classified into three types according to their structures. Type II PKSs are a complex of multiple enzymes and mainly synthesize aromatic polyketides. In this system, a ketosynthase–chain length factor (KS–CLF) complex is most important because it catalyzes decarboxylative condensation of a malonyl-acyl carrier protein (ACP) with an acyl-ACP to synthesize a β-ketoacyl unit. In addition, protein-protein interaction between each enzyme (KS, ketoreductase, dehydratase, or enoyl reductase) and ACP is also important since ACP shuttles an intermediate to each enzyme. Recently, our group found a new class of type II PKS, a highly-reducing type II PKS for polyene biosynthesis, by analyzing ishigamide biosynthesis [1]. Here, we attempted to understand the catalytic mechanism of this highly-reducing type II PKS (IgaPKS) by analyzing the structures of its KS–CLF (Iga11–Iga12) complex in three different states [2].

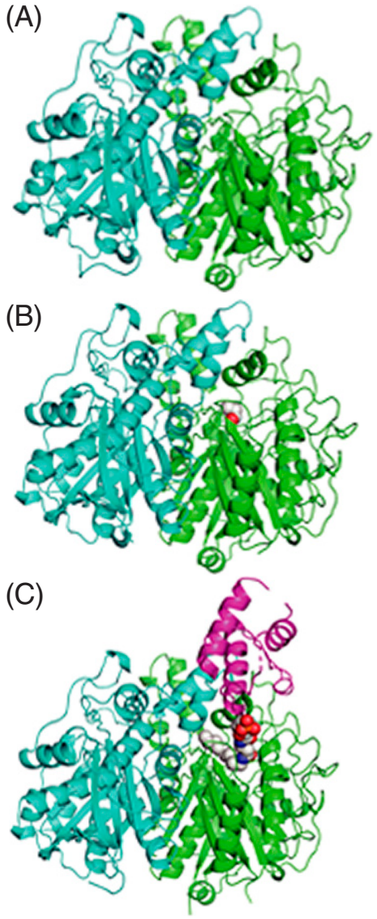

Figure 1: The overall structures of Iga11–Iga12 **(A)**, Iga11–Iga12 binding with a hexanoyl moiety **(B)** and the Iga10=Iga11–Iga12 complex **(C)**. Iga10, Iga11, and Iga12 are shown in magenta, green, and cyan, respectively. The hexanoyl unit and the mechanism-based crosslinker are shown as spheres.

Recombinant Iga11–Iga12 and Iga10 (ACP) were prepared as His-tagged proteins by heterologous expression using *Escherichia coli* BL21(DE3) as a host and purified with Co^{2+} affinity chromatography and gel filtration chromatography. The purified Iga11–Iga12 was used for crystallization. The obtained crystal was subjected to X-ray structure analysis on BL-1A, and the structure was successfully solved by molecular replacement at 1.75 Å resolution [**Fig. 1(A)**]. In addition, hexanoyl-CoA was soaked into the obtained crystal and analyzed by X06SA at Swiss Light Source (SLS), resulting in the structure of Iga11–Iga12 binding with a hexanoyl moiety at the active site (Cys170) at 1.91 Å resolution [**Fig. 1(B)**]. Next, we formed a complex of Iga11–Iga12 and Iga10 by using a mechanism-based crosslinker synthesized by Burkart *et al.* [3] and used for crystallization. The obtained crystal was subjected to X-ray structural analysis at BL-1A, and the structure was successfully solved at 1.98 Å resolution [**Fig. 1(C)**].

The overall structures of Iga11–Iga12 in the three states are highly identical to each other, indicating that Iga11–Iga12 is rigid during the catalysis (**Fig. 1**). The active site of Iga11–Iga12 is mainly surrounded by hydrophobic amino acids [**Fig. 2(A)**]. Interestingly, several polar amino acids are in the center of the active site (e.g., Asp113 and Ser153′; amino acid numbers without prime, with prime and with double prime indicate amino acid residues from Iga11, Iga12, and Iga10, respectively, hereafter). The *in vitro* analysis of the Iga11–Iga12-D113A variant showed that Asp113 was crucial for polyene synthesis by inhibiting

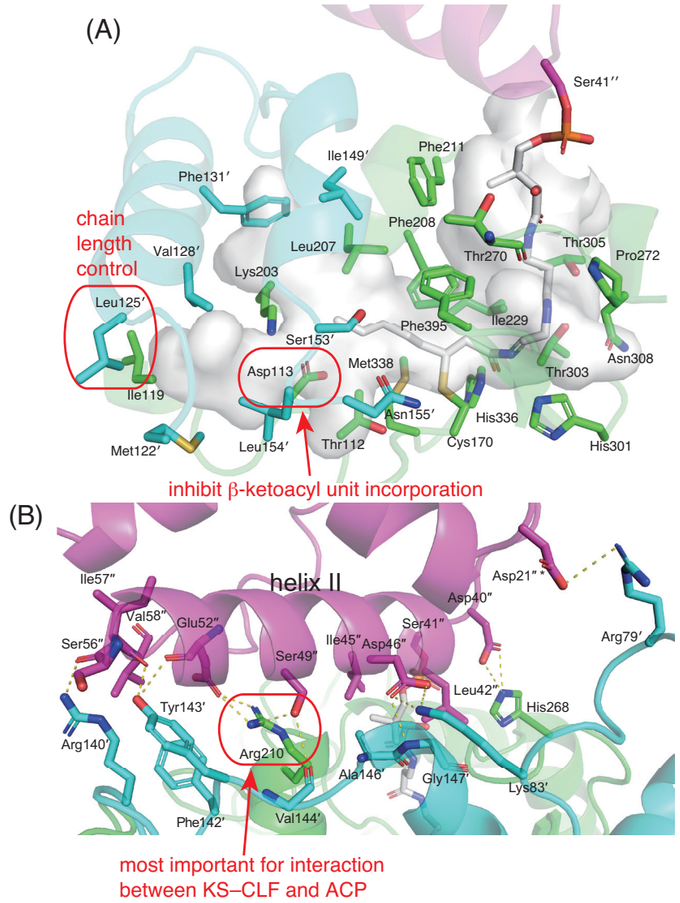

Figure 2: The active site of Iga11–Iga12 **(A)** and the interface between Iga10 and Iga11–Iga12 **(B)**. The white stick indicates the mechanism-based crosslinker used for crosslinking.

a β-ketoacyl-ACP from being a substrate and making Iga11–Iga12 prefer an enoyl-ACP. In addition, Iga11–Iga12-L125′A variant synthesized a polyene with C_{16} while Iga11–Iga12 produced a polyene with C_{14}. This result indicated that the steric wall constituted by Leu125′ controls the chain length of the end product. The structure of the Iga10=Iga11–Iga12 complex showed that helix II of Iga10 (ACP) is most important for the interaction between Iga10 and Iga11–Iga12 [**Fig. 2(B)**]. The interaction was maintained by polar interactions including salt bridges (Arg210-Glu52′′, Lys83′-Asp46′′, and Arg79′-Asp21′′) and hydrogen bonds (e.g., His268-Asp40′′, Arg140′-Ser56′′, [Arg210 and Val144′]-Ser49′′). Analysis of Iga11–Iga12 variants with amino acid substitution at these residues showed that these interactions mediate the formation of the Iga10=Iga11–Iga12 complex. In particular, substitution at Arg210 drastically weakened the interaction, indicating that this residue is most important [**Fig. 2(B)**].

In summary, the structure of the ACP=KS–CLF tripartite complex (Iga10=Iga11–Iga12) in the highly-reducing type II PKS was clarified for the first time. The binding mode of KS–CLF to ACP and how the highly-reducing type II PKS synthesizes a polyene structure and controls the final product chain length were clarified. These results are extremely important for understanding the reaction mechanism of PKS and will provide fundamental information for the artificial design of PKS in the future.

REFERENCES

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