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Structural insight into a novel indole prenyltransferase in hapalindole-type alkaloid biosynthesis

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ABSTRACT

FamD1 is a novel CloQ/NphB-family indole prenyltransferase which involves in hapalindole-type alkaloid biosynthesis. Here the native FamD1 structure and three protein-ligand complexes are analyzed to investigate the molecular basis of substrate binding and catalysis. FamD1 adopts a typical ABBA architecture of aromatic prenyltransferase, in which the substrate-binding chamber is found in the central β-barrel. The indole-containing acceptor substrate is bound adjacent to the prenyl donor. Based on the complex structures, a catalytic mechanism of FamD1 is proposed. Functional implications on the sister enzyme FamD2 are also discussed.

1. Introduction

Aromatic prenyltransferases (APTs) catalyze important enzymatic reactions in both primary and secondary metabolic pathways of bacteria, fungi, and plants. In primary metabolism, membranous APTs which coordinate Mg^{2+} with conserved DxxD motifs to bind PPi moiety of prenyl isoprenoid donor catalyze aromatic prenylation and produce lipoquinones [1]. On the other hand, soluble APTs that lack the Mg^{2+}-coordinating motif are involved in biosynthesis of the scaffold of various microbial secondary metabolites [2]. There are several soluble APTs known to ligate prenyl donors of various lengths to acceptors at various positions on the aromatic rings. Because of the diverse bioactivity of prenylated aromatic metabolites and the substrate promiscuity of APTs, the molecular mechanisms have attracted much interest.

Soluble APTs generally adopt the ABBA-fold that consists of a $(\alpha\beta\beta\alpha)_5$ barrel, in which a large central pocket is formed to accommodate the substrates. Based on sequence identity and structure homology, the APTs are subdivided into two groups. The first group I, also known as the DMATS family enzymes, usually assemble into a homodimer, while the other group II, the CloQ/NphB family, is a monomer. Family I enzymes, also known as indole prenyltransferases, mainly utilize L-tryptophan or its derivatives as the acceptor [3]. These enzymes perform prenylation in a “normal sense” where the primary carbon of prenyl donor adds to the indole ring as well as in a “reverse sense” where the tertiary carbon adds. Family II enzymes utilize phenols, naphthalenes and phenazines as the acceptors and mainly carry out a “normal sense” prenylation. Compared to the extensively studied family I enzymes that a large number of complex structures have been reported, structural information of family II is rather limited that only one ternary complex of NphB [4], one binary complex of CloQ [5] and one apo-form EpzP are available [6].

Hapalindole-type alkaloids including hapalindoles, fischerindoles, ambiguines and welwitindolinones are a large group of cyanobacterial metabolites. These compounds have attracted much attention since discovered in the 1980s due to their wide spectrum of antibacterial, antimycotic, insecticidal, and anticancer bioactivities [7]. More than 80 members have been isolated which possess polycyclic ring systems with complex chiral structures.

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Therefore, intensive effort has been paid in deciphering their biosynthesis pathway [8–15]. The common precursor of these complex molecules has been identified as a geranylated *cis*-indole nitrile (INN) generated by APTs (Fig. 1). Two APTs, named FamD1 and FamD2 (or AmbP3 and AmbP1, respectively), involved in hapalindole and ambiguine biosynthesis in *Fischerella ambigua* UTEX 1903, a cyanobacterium, were characterized [8,13]. FamD2 catalyzes the GPP prenylation of **1** to produce **2** and **3**. Compound **3** is subsequently cyclized by various downstream cyclases to generate products with various polycyclic formations [12–14,16,17]. On the other hand, FamD1 prefers DMAPP as the prenyl donor and turns out **4** and **5** (Fig. 1). The enzyme can also use GPP as a substrate albeit at a lower reaction rate. Moreover, FamD1 can use DMAPP to catalyze a C2 reverse prenylation on hapalindole U to produce ambiguine H [8,13]. Sequence alignment indicates that both FamD1 and FamD2 belong to the CloQ/NphB family but share low identity (<27%) with the other known structures. Notably, FamD1 and FamD2 are highly homologous (51.9% identity), suggesting that their various substrate preference might result from subtle alterations inside the substrate-binding pocket. Therefore, the crystal structures of these novel APTs are of great interest in understanding the biosynthetic mechanism of hapalindole scaffold by revealing the basis of substrate selectivity. In the current study, we report three complex structures of FamD1.

2. Materials and methods

2.1. Expression, purification, and crystallization of FamD1

The gene fragment encoding FamD1 (*Fischerella ambigua* UTEX 1903, GenBank: APB62250.1) was chemically synthesized by GENEray Biotech Co. (Shanghai, China). The fragment was amplified by PCR with forward primer 5′-CGGGATCCGCAGGAGCAGGAGCAATGACCATTGTTAACCGTATTC-3′ and reverse primer 5′-CCCTCGAGTTAGCTCATAACAATACTATTCAGTTTA-3′ and digested by restriction enzymes BamHI and XhoI. The gene fragment was then ligated into the pET28a vector containing 6xHis tag and *Saccharomyces cerevisiae* Smt3p protein (GenBank: AJU61058.1) that are fused to the N-terminal end of the FamD1 protein. The recombinant plasmid was verified by sequencing.

The plasmid was transformed into *E. coli* BL21 (DE3) cells and target protein expression was induced by 0.2 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 16 °C for 18 h. FamD1 purification was carried out at 4 °C as follow. Cells were harvested by centrifugation at 5000xg for 15 min and re-suspended in lysis buffer containing 25 mM Tris-Cl, pH 7.5, 150 mM NaCl and 20 mM imidazole followed by disruption with a French Press. Cell debris was removed by centrifugation at 17,000xg for 1 h. The supernatant was then applied to a Ni-NTA column FPLC system (GE Healthcare). The target proteins were eluted at ~100 mM imidazole when using a 20–250 mM imidazole gradient. Proteins were collected and dialyzed against buffer containing 25 mM Tris-Cl, pH 7.5 and 150 mM NaCl, and 1% v/v SUMO-specific protease (5 mg/mL) overnight to cleave the fusion protein and 6xHis tag. Afterward, protein-containing solution was loaded onto a Ni-NTA column again, and the target protein in the flow-through was collected and concentrated to 20 mg/mL. Protein purity was verified by SDS-PAGE analysis.

The protein FamD1 crystallization condition was screened by using Hampton research screen kit (Hampton Research, Aliso Veijo, CA) and sitting-drop vapor diffusion method. In general, 1 μL protein (10 mg/mL) was mixed with 1 μL of reservoir solution in 48-well Cryschem Plates, and equilibrated against 100 μL reservoir solution at 25 °C. Initial crystals were observed in reservoir solution containing 1.0 M sodium citrate, 0.1 M imidazole, pH 8.0, and reached the size suitable for X-ray diffraction data collection within one month. The crystals were mounted in a cryo-loop and flash-cooled by liquid nitrogen for X-ray data collection. Data sets were collected at beam lines TPS-5A, BL13C1 and BL15A1 of the National Synchrotron Radiation Research Center (NSRRC, Hsinchu, Taiwan) and processed by using HKL2000 program [18]. Prior to structure refinement, 5% randomly selected reflections were set aside for calculating R_{free} [19] as a monitor of model quality.

2.2. Data collection, structure determination, and refinement

The structure of apo form FamD1 was solved by using single isomorphous replacement with anomalous scattering (SIRAS) and

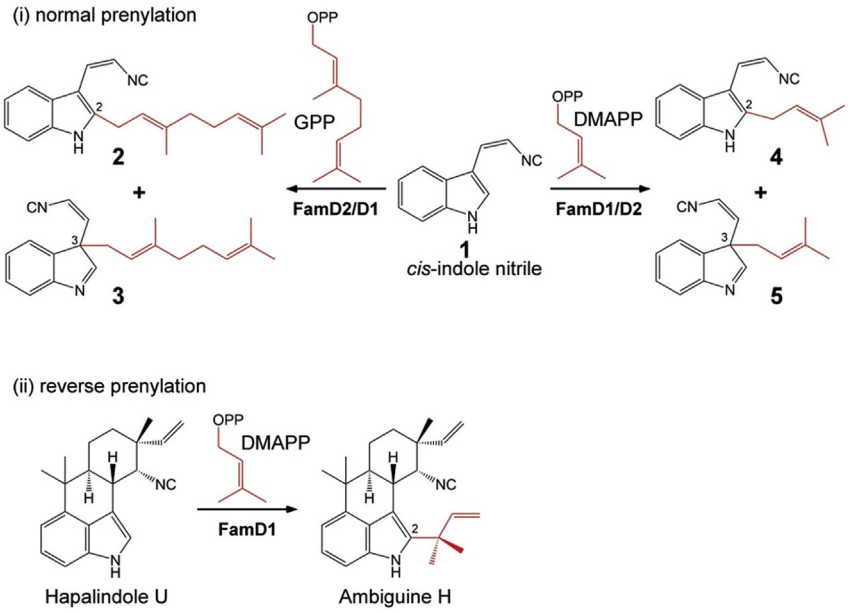

Fig. 1. Normal and reverse prenylation catalyzed by FamD1 and FamD2.