Structural Basis for the Promiscuous Biosynthetic Prenylation of Aromatic Natural Products

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Prenylation is a general term for the chemical or enzymatic addition of a hydrophobic isoprenoid side chain to an accepting molecule (another isoprenoid chemical, small aromatic molecule, protein, etc). Prenylation of aromatic natural products plays a critical role in the biosynthesis of chemically complex and structurally diverse molecules playing important biological functions across a wide phylogenetically diverse group of organisms, from bacteria to mammals. Hybrid natural products such as the anti-oxidant naphterpin[1-3] that contain a polyketide core decorated with 5-carbon (dimethylallyl), 10-carbon (geranyl) or 15-carbon (farnesyl) isoprenoid chains possess biological activities distinct from their non-prenylated aromatic precursors[4]. These hybrid natural products represent new anti-microbial, anti-oxidant, anti-inflammatory, anti-viral and anti-cancer compounds.

Enzymes capable of regiospecific prenylation of bioactive compounds will serve as novel chemoenzymatic tools for natural product diversification and the chemo-enzymatic development of therapeutically novel synthetic compounds.

We recently reported the gene identification, biochemical characterization and high resolution crystal structure of an architecturally novel aromatic prenyltransferase (PTase), Orf2 from *Streptomyces sp.* strain CL190. This protein belongs to a recently identified and completely new class of aromatic prenyltransferases showing no sequence similarity with any protein prenyltransferase[5]. The three dimensional structure of Orf2 consists of a single domain possessing a novel barrel fold (Fig. 1).

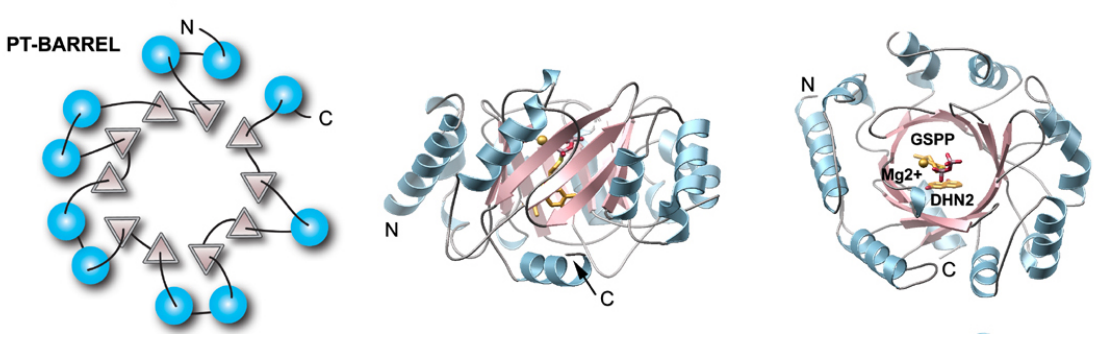

Figure 1. Topology diagram and overall structure of the Orf2 aromatic PT-barrel fold complexed with a non-hydrolysable geranyldiphosphate analog (GSPP), Mg^{2+} and 1,6-dihydroxynaphtalene molecule (DHN2).

This new barrel, termed a PT-barrel for **P**renyl**t**ransferase-barrel, is a cylindrical β-sheet comprising ten anti-parallel β-strands arranged around a central solvent filled core. In an arrangement reminiscent of a TIM-barrel, the cylindrical β-sheet is surrounded by a ring of solvent exposed α-helices; however, the connectivity and directionality of Orf2's secondary structure elements is not shared with the TIM-barrels, β-barrels or even the dimeric α/β-sandwich structural families (Fig. 1).

Using X-ray diffraction data collected at SSRL (BL9.2), ESRF (FIP/BM30A) and BNL (X8C,X6A), we determined four X-ray crystal structures of Orf2 substrate/substrate analog complexes, including Orf2 bound to a TAPS buffer molecule, a binary Orf2 complex containing geranyl diphosphate (GPP) and Mg^{2+}, a ternary Orf2 complex containing a non-hydrolyzable GPP analog, geranyl S-thiolodiphosphate (GSPP), Mg^{2+} and 1,6-DHN, and a ternary Orf2 complex containing GSPP, Mg^{2+} and flaviolin (Fig. 2).

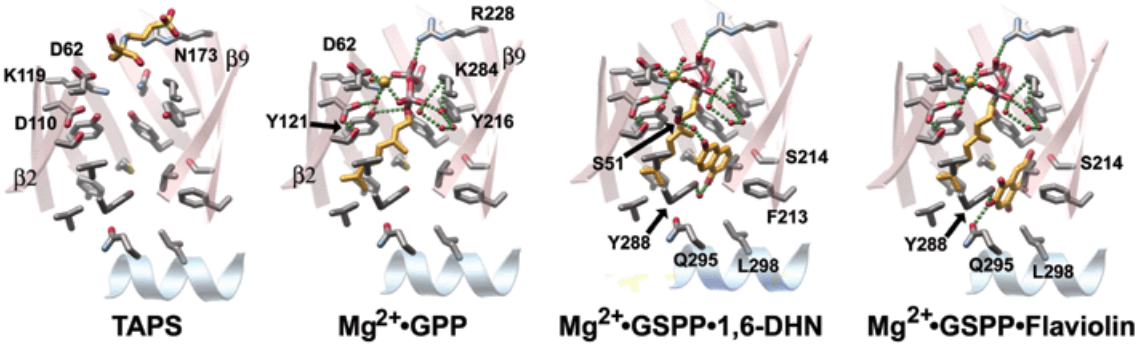

Figure 2. Orf2 substrate/substrate analog complexes. Only the β-strands (minus β-strands 1 and 10) plus the C-terminal α-helix are displayed in the same vertical orientation as in a after a 30° counter-clockwise rotation around the barrel axis. Hydrogen and coordination bonds are shown as green spheres.

Opposite to most previously identified membrane bound aromatic prenyltransferases, this bacterial enzyme is a small soluble monomeric protein. *In vivo*, Orf2 attaches a geranyl group to a 1,3,6,8-tetrahydroxynaphthalene (THN) derived polyketide during naphterpin biosynthesis. *In vitro*, Orf2 catalyzes carbon-carbon and carbon-oxygen based prenylation of a diverse collection of hydroxyl-containing aromatic acceptors of synthetic, microbial and plant origin. This *in vitro* activity against plant derived natural products is the first demonstration of an enzyme activity capable of forming a carbon-oxygen prenyl linkage.

The resultant PTase activity of Orf2 displays promiscuous but regiospecific prenylation activity against diverse aromatic substrates: stilbenes, flavonoids, isoflavonoids and related plant polyketides including naringenin, resveratrol as well as olivetol and olivetolic acid. These later two plant polyketides and their geranylated products serve as intermediates in the biosynthesis of the plant derived polyketide-terpene natural product Δ9-tetrahydrocannabinol (Δ9-THC)[6]. For the isoprenoid diphosphate substrates, Orf2 exhibits no activity with dimethylallyl diphosphate (DMAPP), the highest relative Mg^{2+} dependent activity with geranyl diphosphate and detectable activity with farnesyl diphosphate (FPP).

These crystal structures coupled with *in vitro* assays and the modeling of other known aromatic prenyltransferases provide a basis for understanding and manipulating the regio-specific prenylation of aromatic small molecules using this structurally unique family of aromatic PTases.

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