Structure-Based Engineering of Bacterial Indole Prenyltransferases

Indole prenyltransferases, TleC from *Streptomyces blastmyceticus* and MpnD from *Marinactinospora thermotolerans*, catalyze the reverse prenylation of the C-7 position of the indole ring in (-)-indolactam V using geranyl pyrophosphate or dimethylallyl pyrophosphate to produce lyngbyatoxin A or pendolmycin, respectively. Comparisons of the X-ray crystal structures of TleC and MpnD revealed the intimate structural details of the "reverse" prenylation reactions, and identified the active-site residues governing the substrate specificity for the prenyl donors. Furthermore, structure-guided enzyme engineering successfully altered the preference for the chain length of the prenyl donors, as well as the regio- and stereo-selectivities of the prenylation reactions.

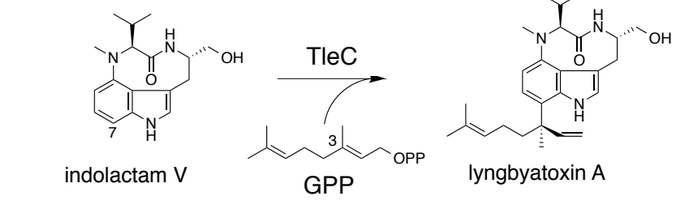
The prenylation reaction catalyzed by prenyltransferases (PTs) is a common enzymatic reaction found in various primary and secondary metabolites [1, 2]. Since the prenylation can lead to significant alteration of biological activities, this reaction is also important for pharmaceutical applications. Indole PTs are one of the subgroups of the aromatic prenyltransferase superfamily that catalyze the electrophilic aromatic substitution of various indoles using prenyl pyrophosphate [3]. TleC and MpnD from *Streptomyces blastmyceticus* and *Marinactinospora thermotolerans* are indole PTs that catalyze the prenylation reactions in the biosynthesis of lyngbyatoxin A and pendolmycin, respectively. These prenylation reactions occur in a "reverse fashion" at the C-7 position of indolactam V (**Fig. 1**) [4, 5].

Interestingly, our in vitro analysis of TleC and MpnD revealed that while each enzyme prefers different prenyl donors, both enzymes are capable of accepting prenyl donors with varying chain lengths (from C_5 to C_{25}). TleC prefers C_{10} geranyl pyrophosphate (GPP) as a prenyl donor, while MpnD prefers C_5 dimethylallyl pyrophosphate (DMAPP). Interestingly, MpnD catalyzes forward prenylation at the C-5 position of indolactam V to produce 5-geranylindolactam V as a major product together with lyngbyatoxin A when GPP is used as the prenyl donor.

To clarify the reaction mechanisms and substrate specificities, we determined the crystal structures of apo TleC, TleC ternary complex structure with indolactam V and DMAPP analogue dimethylallyl S-thiophosphate (DMSPP), apo MpnD, and MpnD ternary complex structure with indolactam V and DMSPP at 1.95, 2.10, 1.60, 1.40 Å resolution, respectively. The overall structures of TleC and MpnD exhibited the ABBA-fold, which consists of ten antiparallel β-strands that assemble into a circular β-barrel surrounded by a ring of solvent-exposed α-helices, observed in the structures of indole PTs (**Fig. 2A** and **2B**).

The substrate-bound structures revealed that the binding modes of indolactam V and pyrophosphate of DMSPP are well conserved, whereas the amino acid residues in the binding sites of the prenyl unit are slightly different between TleC and MpnD. The amino acid residues Trp97, Phe170 and Ala173 in TleC are substituted with Tyr80, Trp157 and Met159, respectively, in MpnD. Inspection of the active site of the complex structure of TleC with substrates showed that the prenyl-donor binding pocket is large enough to accommodate C_{10} GPP as a substrate. The presence of the bulkier Met159 in the active site of MpnD in place of small Ala173 in TleC eliminates the "GPP binding pocket" in MpnD (**Fig. 2C** and **2D**). These observations suggested that these three residues are important for the selectivity of the length of prenyl donor and regioselectivity of the prenylation by TleC and MpnD.

Streptomyces blastmyceticus



Marinactinospora thermotolerans

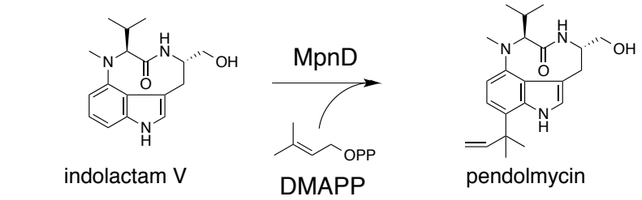


Figure 1: Reaction schemes of TleC and MpnD.

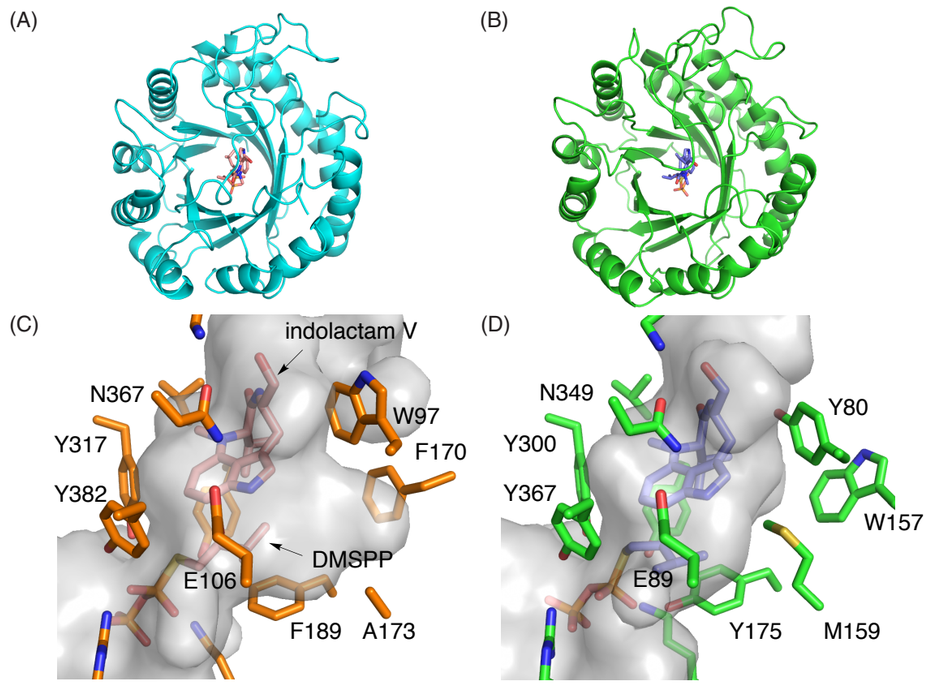


Figure 2: Comparison of the overall structure and active site structures of TleC and MpnD. Overall structures of **(A)** TleC and **(B)** MpnD. Close-up views of the active site cavities of **(C)** the TleC complex structure with indolactam V and DMSPP and **(D)** the MpnD complex structure with indolactam V and DMSPP.

To confirm the importance of these three residues in TleC and MpnD, we performed structure-guided mutagenesis studies. A173M substitution in TleC resulted in the switch in preference toward the smaller DMAPP prenyl donor, while the native GPP prenylation activity decreased dramatically. In contrast, MpnD M159A mutant decreased DMAPP prenylation activity but increased GPP prenylation activity to produce lyngbyatoxin A as a sole product. Furthermore, the double and triple mutants of TleC W97Y/A173M and TleC W97Y/F170W/A173M newly generated C-19-epimer of lyngbyatoxin A (teleocidin A-2) as a major product in addition to lyngbyatoxin A and 5-geranylindolactam V.

In this study, comparison of the crystal structures of the indole prenyltransferases TleC and MpnD clarified the structural details of the enzyme-catalyzed reverse prenylation reactions and active site residues governing the specificity for the length of prenyl donors. Further, structure-guided enzyme engineering successfully altered the preference for the chain lengths of prenyl donors, as well as the regio- and stereo-selectivity of the prenylation reactions. These findings provide not only insight into the catalytic machinery but also strategies for expanding the catalytic repertoire of the enzymes to generate structurally divergent and biologically active novel prenylated indole alkaloids for drug discovery.

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BEAMLINES

BL-17A, BL-5A and AR-NW12A

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