MoeN5: An Important Prenyltransferase Involved in Moenomycin Biosynthesis

Based on the structural basis of MoeN5, it can facilitate the design of new antibiotics.

Moenomycins, first described about 60 years ago, are potent antibiotics because of their ability to inhibit effectively the biosynthesis of bacterial cell walls.[1] Considering the global increase in antibiotic resistance, Moenomycin A (MmA, **1**; **Fig. 1**), a member of the moenomycin family, has become a renewed target for the further development of MmA analogues because of its poor pharmacokinetic property.

In the MmA biosynthesis, 17 enzymes participate in the complicated process. **Figure 1** clearly shows that MoeN5 catalyses the reaction of geranyl diphosphate (GPP, **6**) with the cis-farnesyl group in phosphoglycolipid (FPG trisaccharide, **5**) to produce (C_{25}) moenocinyl-sidecahin-containing lipid **7**. (C_{25}) moenocinyl trisaccharide **7** subsequently undergoes further transformation to give rise to the formation of **1**. Among the biosynthesis paths, the authors in this report have solved the crystal structure of MoeO5 (involved in reaction **3**→**4**);[2] they also attempted to determine the X-ray structure of MoeN5. To elucidate the mechanism of the uncommon *head-to-middle* prenyltransferase, they proceeded to determine the structures of MoeN5 in complexes with GPP, FPG and a series of model glycolipids.

No similar related structures were identified after a Basic Local Alignment Search Tool (BLAST) search. A collaborative team led by Rey-Ting Guo (Tianjin Institute of Industrial Biotechnology) hence solved the structure of MoeN5 using single-wavelength anomalous diffraction (SAD) with a 3.3-Å Se-Met data set at **TLS 13B1** and **TLS 15A1**.[3] After an iterative model refinement, several missing regions were lacking. It was difficult to propose a catalytic mechanism because of the poor resolution. To overcome this problem, they attempted to use four fusion protein tags, including thioredoxin from *Escherichia coli*, SUMO (small ubiquitin-like modifier) from *Saccharomyces cerevisiae*, Sac7d (chromosomal protein 7d) from h*yperthermophiles Sulfol-*

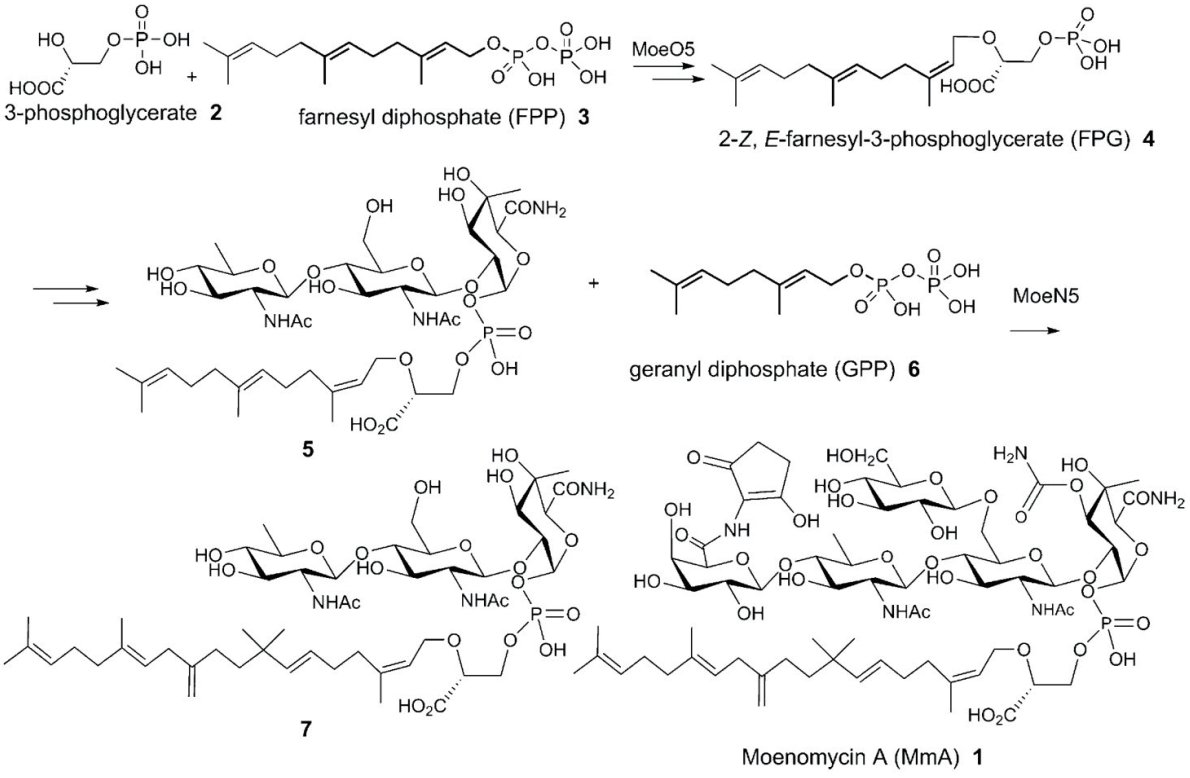

Fig. 1: Selected steps in the biosynthesis of moenomycin A. [Reproduced from Ref. 3]

obus acidocaldarius and Sso7d (chromosomal protein 7d) from *Sulfolobus solfataricus*, to obtain improved crystal forms. Among the four constructs, the MoeN5-Sso7d construct was crystallized in two space groups; both crystals yielded the improved data sets, 2.29 Å for the C222 form and 2.8 Å for the I222 form. The full structure of MoeN5 was eventually interpretable because the electron-density map was improved significantly with the two superior data sets.

Two features were observed in the overall structure of MoeN5-Sso7d – MoeN5 has an all α-helix structure, and two monomers in an asymmetric unit form a dimer (**Fig. 2(a)**). For clarity, size-exclusion chromatography with multi-angle laser scattering (SEC-MALS) was performed to prove that the dimer form is a natural state unaffected by the addition of Sso7d. Use of the PDBeFold server revealed that the MoeN5 structure shared some similarities with head-to-tail trans-prenyl synthases, including a (C_{10}) geranyl transferase from *Thermotoga maritima*, a (C_{20}) geranylgeranyl diphosphate synthase from *Sinapis alba*, and a polyprenyl diphosphate synthase from *Shigella flexneri*. These synthases contain two Asp-rich (DDXXD or DXXXD) domains; abbreviation names FARM and SARM stand for the first and second Asp-rich domain, respectively (**Fig. 2(b)**). In the same manner, MoeN5 also had two domains. The collective evidence indicated that the active site is expected to be near the two Asp-rich domains.

The authors subsequently proceeded to locate the two substrate-binding sites, S1 (for GPP **6**, binding) and S2 (for FPG-trisaccharide **5**, binding), through co-crystallization, ligand soaking and structural analysis. Based on the results, the GPP was located exactly in the S1 position (**Fig. 2(c)**), but the FPG case for the S2 position was unsuccessful. To address this problem, the authors executed an alternative strategy to solve the complex structures of MoeN5 with a series of glycolipid models, which turned out to be useful in mapping the binding pocket. This structural information indicated that FPG-trisaccharide adopted a bent C-terminus conformation for further catalytic reaction upon substrate binding (**Fig. 2(d)**). The proposed catalytic mechanism comprises four steps: i: phosphoglycolipid **5** (blue) binds to S2 with its bent (C6-11) side chain and GPP **6** is shown (yellow); ii: diphosphate is released from GPP **6** and attack by C6,7/C10,11 in **5** yields the cyclopentyl carbocation **13**; iii: **13** rearranges to cyclohexyl carbocation **14**; iv: removal of a methyl proton in **14** by PPi yields product **7** (**Fig. 2(e)**).

In summary, the authors have achieved the first structure of MoeN5, an important prenyltransferase participating in MmA biosynthesis. This structural information can, importantly, provide a future design of MmA analogues as new lead compounds; the use of small fusion tags is applicable to other cases and is expected to improve the quality of diffraction data. (Reported by Chun-Hsiang Huang)

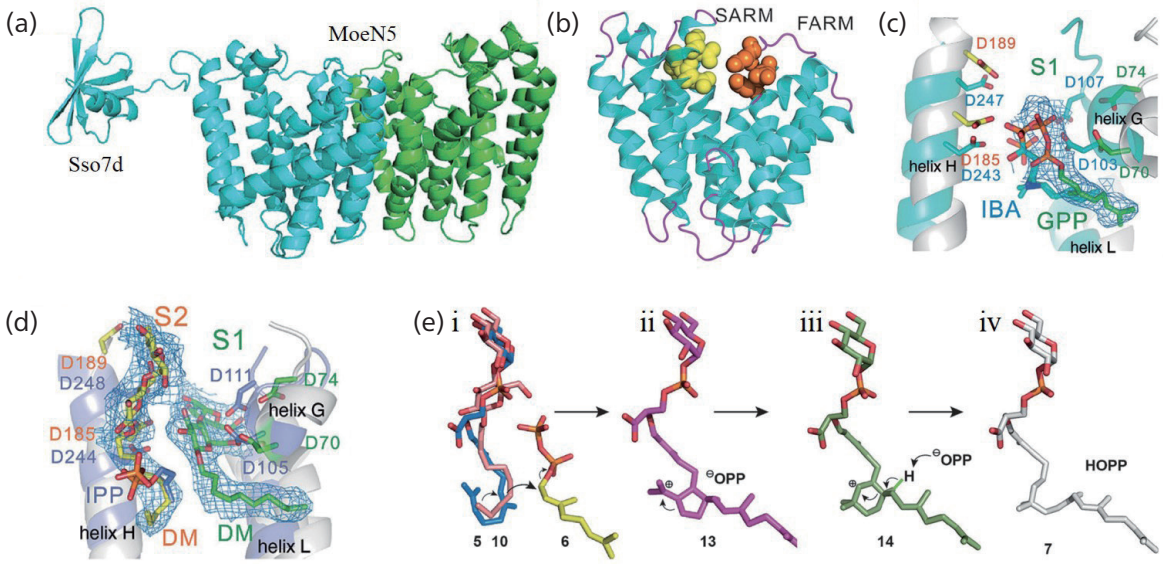

Fig. 2: Overall structure, position of binding site and proposed catalytic mechanism of *Streptomyces ghanaesis* MoeN5. (a) Dimer form of MoeN5. The Sso7d tag is ordered in only one molecule in the dimer. (b) Position of two Asp-rich domains. (c) S1 binding site. (d) S2 binding site. (e) Proposed catalytic mechanism [Reproduced from Ref. 3]

This report features the work of Rey-Ting Guo and his co-workers published in Angew. Chem. Int. Ed. ***55****, 4716 (2016) .*

TLS 13B1 SW60 – Protein Crystallography
TLS 15A1 Biopharmaceuticals Protein Crystallography
- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Sciences

| References |

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How the Fidelity of DNA Repair Is Modulated in Human Beings

The crystal structures of DNA Polymerase λ in various states were determined to elucidate the structural mechanism for the fidelity modulation.

The mechanism of DNA polymerase (pol) fidelity is of fundamental importance in chemistry and biology. Whereas the pols responsible for DNA replication are required to perform catalysis with great fidelity, those involved in DNA repair or mutagenic functions typically exhibit less fidelity. Although high-fidelity pols have been well studied, much less is known about how some pols achieve medium or low fidelity with functional importance. A structural basis for an atypical dG:dGTP mismatch (GG mismatch) incorporated catalyzed by the most error-prone DNA polymerase from Africa swine fever virus (Pol X) was reported.[1] Pol X catalyzes the dG:dGTP mismatch on prebinding syn-dGTP in the absence of DNA; the syn-dGTP then form an anti:syn dG:dGTP Hoogsteen base pair with the template dG of the DNA. His115 was found to be the critical residue in stabilizing the syn-dGTP; mutation of His115 to an alanine residue abolished the syn-dGTP (only anti-dGTP observed), and resulted in a fidelity increase by 330 fold. This unprecedented finding of pre-binding MgdNTP was in contrast to the paradigm in DNA polymerases, which states that DNA binding precedes that of MgdNTP.

Wen-Jin Wu, Ming-Daw Tsai and their co-workers of Academia Sinica extended their investigation to human DNA polymerase λ Pol λ.[2] They examined how Pol λ achieves its moderate fidelity by determining 12 crystal structures of *apo*-Pol λ, MgdTNP binary complexes, MnMgdNTP binary complex (in which dNTP refers to dGTP dATP, dTTP and dGTP), dG:dATP mismatched ternary complex, *apo*-L431A mutant, binary complexes of L431A:MgdCTP, L431A:MgdTTP and L431A:dGTP, dG:dCTP matched ternary complex but with L431A mutant. X-ray diffraction data were collected at **TLS 15A1**, **TLS 13B1**, **TLS 13C1** and **SP 44XU**. The authors also performed pre-steady-state kinetic analyses to determine the rate of dNTP incorporation, apparent K_d values of incoming dNTP to Pol-DNA binary complexes, catalytic specificity and fidelity. They showed that *apo*-Pol λ already exists in the closed conformation (**Fig. 1(a)**), unprecedentedly with a preformed MgdNTP binding pocket (**Figs. 1(c)-1(e)**), and binds MgdNTP readily in the active conformation in the absence of DNA (**Fig. 1(d)**). A large conformational change occurs upon the binding of the gapped DNA substrate containing a 5′-phosphate in the downstream primer (**Fig. 1(f)**).

The structure of Pol λ:MgdNTP binary complexes revealed that the tight MgdNTP binding was contributed in part from partial stacking between the Tyr505 side chain and the incoming dNTP. The MgdNTP affinity was decreased significantly in the Y505A mutant