Y160A, Y160F and Y160W, to conduct activity assays. Notably, the result shows distinct effects on ZEN and α-ZOL. In regard to ZEN, compared with the wild type, all mutants except Y160F having equal activity possess less ZEN-hydrolyzing activity, which indicates that Y160 is the most suitable residue for ZEN interaction (**Fig. 2(c)**). As regards α-ZOL, compared to the wild type, only Y160A exhibits an increase, more than 70%, in α-ZOL hydrolyzing activity (**Fig. 2(c)**). The complex structure of Y160A/α-ZOL indicates that the unfavourable close contact (the side chain of Y160) disappears; Y160A thus makes an active-site environment suitable for α-ZOL binding and hydrolysis, which conclusion is supported by data from isothermal titration calorimetry (ITC).

In summary, Guo and his collaborators determined the crystal structures of ZHD101 and RmZHD. Comparison of the two enzyme structures clearly shows that two vital structural differences (the β6-α5 loop and Y160) are involved in the catalytic reaction. These results provide the important molecular mechanism of substrate binding and catalysis for the ZHD family of enzymes; this critical information can be applied for further protein engineering and genetic modification to diminish the mycotoxin. (Reported by Chun-Hsiang Huang)

This report features the work of Rey-Ting Guo and his collaborators published in ACS Catal. ***8****, 4294 (2018).*

TPS 05A Protein Microcrystallography
TLS 13C1 SW60 – Protein Crystallography
TLS 15A1 Biopharmaceuticals Protein Crystallography

- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Science

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AmbP3: A Proficient Enzyme Involved in the Normal and Reverse Prenylation of Hapalindoles

Compounds related to hapalindole can serve as potential pharmaceutical leads. This study is the first to elucidate how a single enzyme catalyses normal and reverse prenylation of hapalindoles. These findings provide vital clues to the engineered biosynthesis of prenylated natural products.

Hapalindoles, produced from Cyanobacteria, are bioactive secondary metabolites with diverse chemical structures; compounds related to hapalindole are considered to have pharmacological effects, such as antibacterial, antifungal, insecticidal and antimitotic activities.[1,2] Prenylation is a crucial reaction to increase the chemical complexity of these compounds; namely, the prenylated compounds typically improve the bioactivities because the prenyl moiety makes the compound more lipophilic. In general, the prenylation of two types, normal and reverse, is performed by prenyltransferases (PTases). It is hence important to understand the catalytic mechanism of PTases for future engineering approaches to synthesize unnatural bioactive compounds.

Some PTases, including DMATS (dimethylallyl tryptophan synthase), Fnq26 and TleC, conduct normal or reverse prenylation depending on the substrates, which means that these PTases have the potential for plasticity of substrate binding. No structural data were, however, available to elucidate how a single enzyme switches the two distinct prenylation modes upon different substrates binding. For this reason, a research team led by Ikuro Abe (Graduate School of Pharmaceutical Sciences, University of Tokyo) determined the structure of *Fischerella ambigua* AmbP3 alone and complex structures of AmbP3/DMSPP (DMSPP is a DMAPP analogue), AmbP3/DMSPP/hapalindole U (reverse pathway in **Fig. 1(a)**) and AmbP3/DMSPP/hapalindole A (normal pathway in **Fig. 1(b)**). All diffraction data sets were collected at **TLS 15A1**.[3]

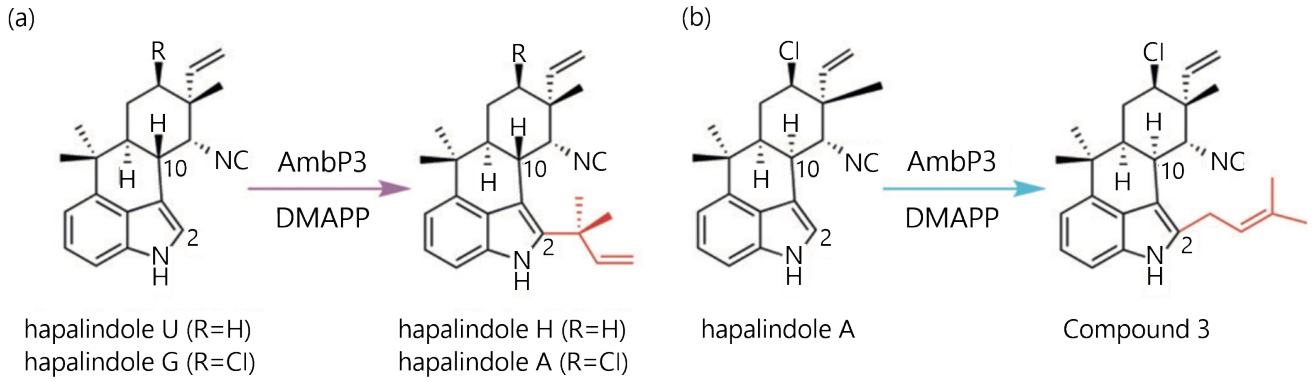

Fig. 1: AmbP3-mediated prenylation of hapalindoles. (a) Reverse prenylation of hapalindole U (HU) or G (HG) with dimethylallyl pyrophosphate (DMAPP) to yield ambiguine H or A. (b) Normal prenylation of hapalindoles A (HA) to yield compound 3. [Reproduced from Ref. 3]

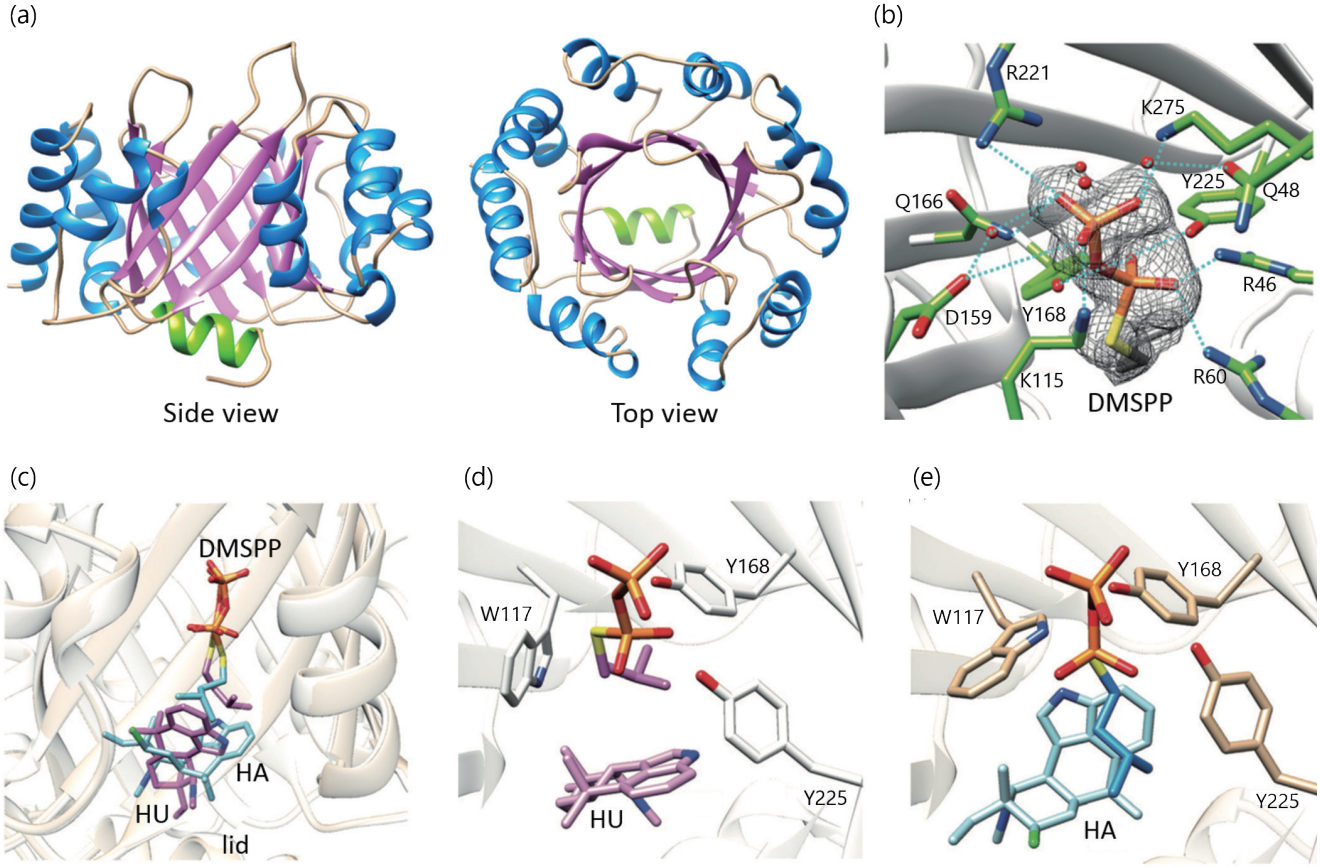

Fig. 2: (a) Overall structure of AmbP3. The central circular β-barrel is in magenta, outer α-helices blue and the bottom "lid" green. (b) Interaction network of DMSPP. Dashed blue lines indicate hydrogen bonds, red spheres water molecules. (c) Superimposition of HU (purple) and HA (cyan). (d) Substrate binding mode of HU. (e) Substrate binding mode of HA. [Reproduced from Ref. 3]

Figure 2(a) shows that the overall structure of AmbP3 comprises three regions, including central circular β-barrel, outer α-helices and the bottom "lid." Structural analysis indicates that, similar to other reported PTases, the top two-thirds of the β-barrel cavity ispolar area; the area otherwise near the bottom "lid" is non-polar. In AmbP3/DMSPP, DMSPP is located at the top of the β-barrel cavity (polar area) to support the α- and β-phosphates *via* hydrogen bonds with water molecules and protein polar residues (**Fig. 2(b)**). Through the phosphate binding mode, the prenyl moiety can extend down the bottom "lid" (**Fig. 2(c)**). Among these polar residues, Tyr168 and Tyr225 form also a "tyrosine shield" to stabilize the carbocation intermediate during the catalysis (**Fig. 2(b)**).

In **Fig. 2(c)**, a comparison of the two ternary structures (AmbP3/DMSPP/HU or HA) clarifies the binding modes of the prenyl acceptors (HU and HA) and prenyl donors (DMSPP). In both cases, notably, the HU and HA are held in the same hydrophobic regions, but they adopt orientations completely different for reverse and normal prenylation, respectively (**Fig. 2(d) and 2(e)**). Comparison with several published PTases with broad substrate specificities indicates that the terpenoid moiety of hapalindoles is more important for substrate recognition than the indole moiety.

The subsequent study focused on the prenyl donor. In the AmbP3/DMSPP/HU, the dimethylallyl group interacts with the indole of HU, Trp117 and Tyr168 (**Fig. 2(d)**); in the AmbP3/DMSPP/HA, the same group is surrounded by the indole of HA, Trp117 and Tyr225 (**Fig. 2(e)**). The combination of these effects indicates that Trp117 is involved in stabilization of the cation intermediate. To test the importance of Trp117, three mutants – W117F, W117Y and W117A –were constructed for prenylation assays. All mutants completely lost their activity, except W117Y (94% for HU and 74% for HA *vs*. wild-type enzyme). These data not only confirm that Trp117 is an important catalytic residue but also reflect the plasticity of the active site of AmbP3 according to the W117Y case. Comparison of AmbP3 and two ternary structures shows that AmbP3 provides sufficient space for the hapalindoles and DMSPP in differentorientations because of the conformational change of Trp117; this observation is consistent with the results from reported PTases, such as CloQ and EpzP.

In summary, the structural information and biochemical data enable two major conclusions based on the excellent model. (1) Because of the hydrophobic substrate binding pocket, AmbP3 is capable of accommodating two separate hapalindoles. (2) The tolerance of AmbP3 for the allyl group of the prenyl donor in different orientations is attributed to the aromatic residues that can form a cation shield. This report is the first to offer structural information for the normal and reverse prenylations catalysed by a single enzyme; these findings become applicable to the future engineering of PTases to regulate the prenylation pathways (normal or reverse) to produce bioactive unnatural compounds. (Reported by Chun-Hsiang Huang)

This report features the work of Ikuro Abe and his collaborators published in Angew. Chem. Int. Ed. Engl. ***57****, 560 (2018).*

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