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X-Ray Crystal Structure Analysis of Pseudoglycosyltransferase VldE

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1 Introduction

VldE is a pseudoglycosyltransferase involved in the biosynthesis of validamycin A, a naturally occurring trehalose inhibitor that is used as antifungal agent to protect crop [1]. VldE catalyzes a nonglycosidic C-N bond formation between GDP-valienol and validamine 7-phosphate to produce validoxylamine A 7'-phosphate (VDO) (Figure 1), an intermediate of validamycin A. Previous mutagenesis experiment of VldE suggested that Asp158 and His182 play the pivotal role in catalyzing the formation of VDO [2]. However, the mechanism employed by VldE to catalyze the non-glycosidic C-N bond is remained unclear. Hence, the crystal structures of D158N and H182A mutants were elucidated in order to further clarify the underlying catalytic mechanism of VldE.

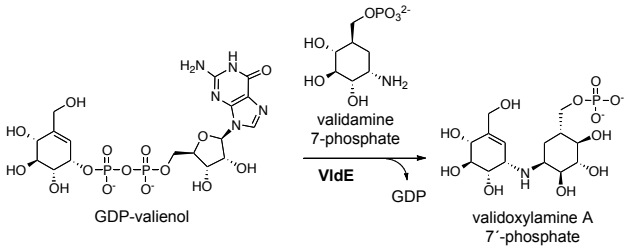

Figure 1. Proposed reaction catalyzed by VldE.

2 Materials and Methods

Crystallization – Diffraction-quality crystals of VldE D158N were obtained after 2 days of incubation at 20 ˚C, in 100 mM Tris-HCl (pH 8.5) containing 26% PEG4000, with 10 mg/mL of purified VldE D158N solution. While the crystals of H182A were obtained after a week on incubation at 4 ˚C, in 100 mM Tris-HCl (pH 8.5) containing 26% PEG4000, with 10 mg/mL of purified VldE H182A solution. Both crystals were obtained by using sitting-drop vapor-diffusion method. *Data collection* – Both type of crystals were transferred into the soaking solution with 15% (v/v) ethyleneglycol for 10 sec for cryoprotection and then flash cooled at -173°C in a nitrogen-gas stream. The X-ray diffractions of crystals were collected at BL-1A, processed and scaled with *XDS*. The structure was solved by the molecular replacement method with *Phaser-MR (simple one-component interface)* using pseudo-glycosyltransferase (VldE, PDB accession code: 3VDN) as search model. The structure was modified manually with *Coot* and refined with *PHENIX*.

3 Results and Discussion

The crystal structure of VldE D158N and H182A were solved at 2.5 Å and 2.4 Å, respectively. The final *R*-value was 24.2% (R_{free} = 28.4%) for VldE D158N and 18.8% (R_{free} = 24.0%) for VldE H182A. The mutants VldE retains the wild-type homodimeric overall structure, where each monomer consists of two Rossman $\beta\alpha\beta$ domains (Figure 2) [2] .

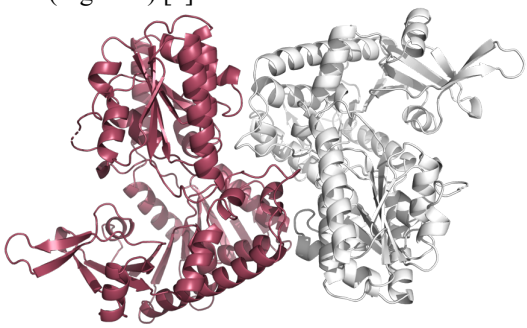

Figure 2. Overall structure of VldE H182A.

The electron density map of D158N and H182A and the elucidated structure of both mutants confirmed that Asp158 was successfully converted to Asn158, and His182 to Ala182, respectively (Figure 3). The crystals structures of these mutants complexed with the substrates, GDP-valienol and validamine 7-phosphate, and the enzymatic product VDO will be prepared to fully unraveled the importance of Asp158 and His182 for VldE catalytic mechanism.

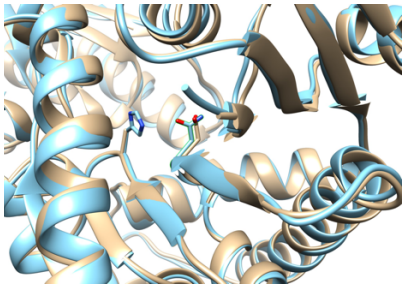

Figure 3. Close-up view on Asn158 and Ala182 residues. D158N and H182A mutant is represented cyan and gold, respectively.

References

[1] T. Iwasa, H. Yamamoto, M. Shibata, *J. Antibiotic*. 24, 107-113 (1971)
[2] M.C. Cavalier, Y.S. Yim, S. Asamizu, D. Neau, K.H. Almabruk, M. Taifo, Y.H. Lee, *PLOS ONE*, e44934 (2012)

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