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X-ray Crystal Structure Analysis of Indole Prenyltransferase from *Aspergillus fumigatus*

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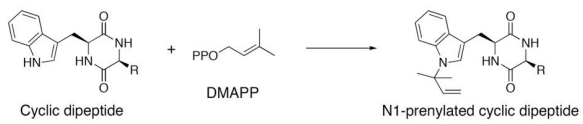
1 Introduction

CdpNPT from *Aspergillus fumigatus* is an indole prenyltransferase (PT) that catalyzes reverse prenylation at position N1 of tryptophan-containing cyclic dipeptides with dimethylallyl diphosphate (DMAPP) as the prenyl donor (Scheme 1) [1]. The enzyme also shows varying substrate specificities and regiospecificities for the prenylation of the indole core and the prenyltransfer reactions classified into two types: 'regular' prenylation (attack at the primary centre of the dimethylallyl carbocation) and 'reverse' prenylation (attack at the tertiary centre). Recent structural analysis of this enzyme has suggested that the varying substrate specificities and regiospecificities for the prenylation of this enzyme are derived from dynamic motion of active site structure of the enzyme [1].

To further clarify the structure–function relationship of this enzyme and to understand the intimate structural details of the enzyme-catalyzed processes, we expressed a truncated (38–440) His6-fused *A. fumigatus* CdpNPT (CdpNPTΔN) in *Escherichia coli* and determined X-ray crystal structure of CdpNPTΔN at PF.



Scheme 1: Prenylation of tryptophan-containing cyclic dipeptides by CdpNPT.

2 Materials and Methods

Crystallization –20 mg/mL CdpNPTΔN mutant was incubated with 5 mM L-Trp on ice for 30 min, and crystallized using sitting-drop vapour-diffusion method. Crystals were generated in 13 µl sitting-drops containing 2 µl of protein, 10 µl of mother liquor equilibrate, and 1 µl of 5% (w/v) *n*-dodecyl-*N*,*N*-dimethylamine-*N*-oxide over 500 µl of reservoir solution at 5°C. CdpNPTΔN was crystallized in buffer solutions containing 0.1 M Tris-HCl (pH 8.3 – 8.6) and 1.26 – 1.40 M ammonium sulfate after a few days incubation.

Data collection – Crystals were transferred into a solution of 20% (v/v) glycerol in the mother liquor, and were flash-frozen in a nitrogen stream. The X-ray diffractions of crystals were collected at BL5A, and were processed and scaled with *XDS*. The structure was solved by the molecular replacement method with *Molrep* in the *CCP4* suite using CdpNPT (Protein Data Bank accession no. 4E0T) [1] as the search model. The structure was modified manually with *Coot* and refined with *PHENIX*.

3 Results and Discussion

X-ray structure of CdpNPTΔN was solved by the molecular-replacement method and was refined at 1.91 Å resolution (Figure 1). The final *R*-value was 18.6% (R_{free} = 21.3%). Asymmetric unit contains four molecules and three molecules are well superimopasable, with an r.m.s.d. of 0.22 - 0.38 Å for backbone Cα atoms.

Comparison of the apo CdpNPT and previously reported substrate-bound CdpNPT structures demonstrated that there is no significant structural change between structures (r.m.s.d of less than 0.38 Å). Further struture analysis for the substrate promiscuity of this enzyme are now in progress.

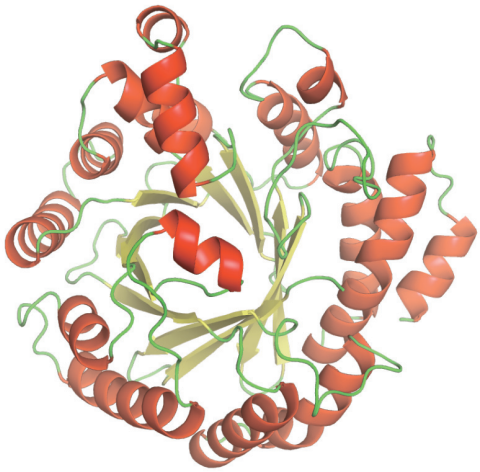


Fig. 1: Overall structure of CdpNPTΔN. Helix, strand, and loop are colored in red, yellow and green, respectively.

Acknowledgement

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References

[1] Schuller, J. M. *et al*., J. Mol. Biol. **422**, 87-99 (2012).

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