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X-ray crystal structure analysis of fungal indole prenyltransferase with β-carbolines

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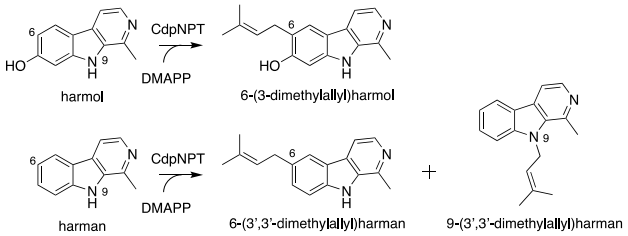
1 Introduction

β-Carboline is a valuable indole alkaloid produced by a variety of organisms, and it can be found in several food items, alcoholic beverages, and tobacco smoke, as well as human tissues and body fluids. Hence, we focused on studying the indole prenyltransferase CdpNPT to introduce more diversity into β-carbolines. CdpNPT, originally identified in *Aspergillus fumigatus* and *Neosartorya fischeri*, is highly substrate-tolerant and accepts various compounds containing indole rings as prenyl acceptors with dimethylallyl diphosphate (DMAPP) as the prenyl donor to catalyze prenylation reactions [1, 2]. We have shown that CdpNPT can accept the β-carbolines harmol and harman as prenyl acceptors, forming corresponding prenylation products (Scheme 1) [3, 4]. Therefore, in order to gain more insights into the detailed mechanism behind these reactions, we performed a crystal structure analysis of CdpNPT complexed with both compounds.



Scheme 1: Substrates and product of CdpNPT.

2 Experiment

Crystallization – Diffraction-quality crystals of the *C*-His$_6$-tagged CdpNPT (38-440) with 2 mM harmol or harman were obtained at 20 ˚C, in 200 mM ammonium phosphate dibasic and 20% (w/v) PEG3350 (pH 8.0) with 10 mg/mL of purified CdpNPT solution, by using sitting-drop vapor-diffusion method.

Data collection – The crystals were transferred into the soaking solution with 20% (v/v) glycerol 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 BL1A, processed and scaled with *XDS* and *AIMLESS* in the *CCP4* program package. The structures were solved by the molecular replacement method with *Phaser-MR* using CdpNPT crystal structure (PDB entry 4E0U) as a template. The structures were modified manually with *Coot* and refined with *PHENIX*.

3 Results and Discussion

The crystal structures of CdpNPT complexed with harmol and harman were solved by X-ray crystallography at 2.40 Å and 2.43 Å resolution, with the final *R*-value of 18.4% (R_{free} = 22.3%) and 21.8% (R_{free} = 23.6%), respectively. Harmol and harman were observed in the reported typical prenyl acceptor-binding region in the active-site cavity within the central $(\alpha\alpha\beta\beta)_5$ barrel and displayed several π stacking interactions in the identical position (Fig. 1). The catalytic Glu116 residue forms a hydrogen bond interaction with the N-9 atom in the indole core of both molecules, leading to the proposed prenylated mechanism initiated by the proton abstraction of the indole amine by Glu116.

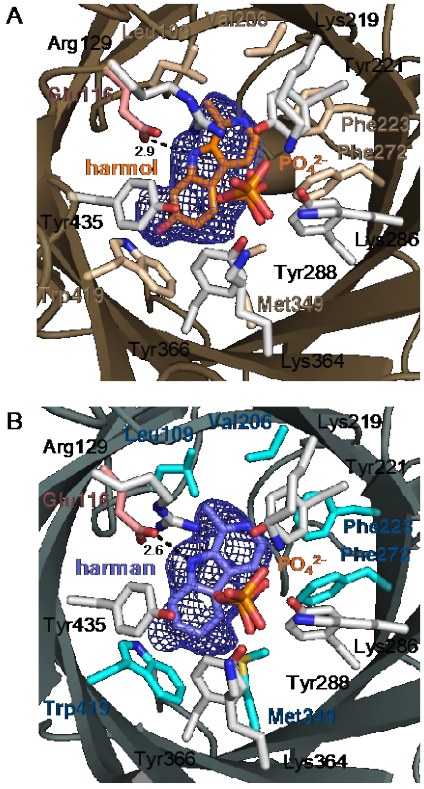


Fig. 1: Close-up view of the CdpNPT-harmol active site (PDB code: 7XVJ) (A) and CdpNPT-harman active site (PDB code: 7Y3V) (B), with a representative OMIT electron density map (mFo-DFc) contoured to 4σ around harmol and 3σ around harman, respectively. The dashed lines represent the distances in Å.

Acknowledgement

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References

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