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Structural basis for the diterpene cyclization in cyclooctatin biosynthesis

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Introduction

Cyclooctatin is a potent inhibitor of lysophospholipase, which catalyzes the hydrolysis of the fatty acid ester bonds of lysophospholipids. This inhibitor was isolated from the broth of *Streptomyces melanosporofaciens* MI614-43F2 while screening for lead compounds for the development of anti-inflammatory drugs targeting lysophospholipase.

Cyclooctatin has a unique tricyclic diterpene skeleton characterized by a 5-8-5 fused ring system (Fig. 1A). In the biosynthesis of cyclooctatin, the biosynthetic gene product CotB1 synthesizes geranylgeranyl diphosphate (GGDP) from isopentenyl diphosphate and dimethylallyl diphosphate, and then CotB2 catalyzes the stereospecific cyclization of GGDP to give cycloocta-9-en-7-ol (Fig. 1B). Next, the cytochrome P450, CotB3, likely catalyzes the stereospecific hydroxylation of cycloocta-9-en-7-ol at C-5 to form cycloocta-9-en-5,7-diol. Finally, the other cytochrome P450, CotB4, catalyzes the hydroxylation of cycloocta-9-en-5,7-diol at C-18 to yield the final product, cyclooctatin. Although cyclization is a key reaction in cyclooctatin biosynthesis, no 3D-structural information has been available to date. To clarify the cyclization mechanism of CotB2, we determined the crystal structure of CotB.

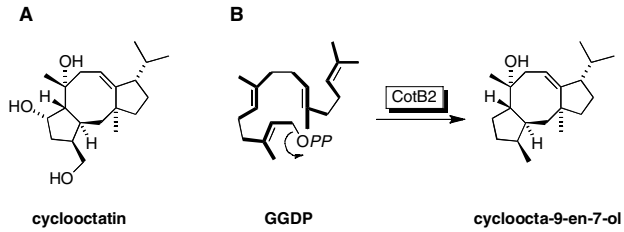

Fig. 1 Chemical structure of cyclooctatin (A), and the cyclization catalyzed by a diterpene cyclase, CotB2 (B).

Materials and Methods

Crystallization - Crystal of native CotB2 was obtained by the hanging-drop vapor-diffusion method. The reservoir solution contained 0.1 M HEPES-NaOH (pH7.0-8.5) and 1.4-1.5 M ammonium formate. Crystal of SeMet substituted CotB2 was obtained in the reservoir contains 0.1 M Tris-HCl (pH8.0) and 2.2 M ammonium formate.

Data collection and processing – Prior to data collection, the CotB2 crystal was flash-annealed to improve the mosaicity and highest resolution. The X-ray diffraction data of native proteins was collected using the beamline NW-12 at PF. SAD data set of SeMet substituted CotB2 was collected using beamline NW-12. The image sets were integrated and scaled using HKL2000.

Results and Discussion

Overall structure of CotB2 - The crystal structure of Se-Met substituted CotB2 was determined for the first time as a diterpene cyclase by the SAD method and crystal structure of native CotB2 was refined at 2.4 Å resolution. CotB2 is composed of a terpene cyclase fold (Gly9-Asn292), forming 13 α-helices. The electron density of N-terminal and C-terminal residues of CotB2 (Met1-Ala8 and Lys293-Gln307) was not observed, indicating that this region is disordered in the crystal. CotB2 forms a dimer. The α6, α7, α8, α10, and α11 helices form dimer interface with buried surface of 3,424 Å^2. Dali search for structurally related proteins retrieved aristorochene synthase (AS) from *Aspergillus terreus*, which is one of the sesquiterpene cyclase, with highest structural similarity (Z-score = 17.0, RMSD of Cα atoms = 3.6 Å).

Insight into catalytic mechanism and cyclization – The structure of CotB2 takes an open form with its volume of active site pocket of 937 Å^3. The ^{110}DDMD motif and ^{220}NDFYSYDRE motif, which are predicted to be involved in catalytic process, are faced at the entrance of the active site pocket (Fig. 2). The structural comparison with AS from *A. terreus* provides insight into the catalytic mechanism of CotB2. Asp111 in ^{110}DDMD motif is predicted to form ion pair with Arg294 to close the active site pocket. Then, Tyr315 enters the active site and promotes the ionization of GGDP. Asp110, Asn220, and Ser224 stabilize Mg^{2+} ion for binding of the diphosphate moiety of GGDP. Asn220 might be involved in protonation of a reaction intermediate. The depth of the active site is formed by many hydrophobic and aromatic amino acid residues, which are thought to be involved in recognition and cyclization of GGDP.

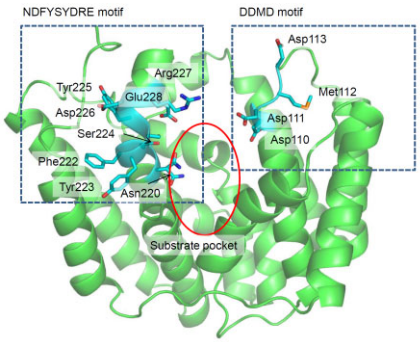

Fig. 2 Monomer structure of CotB2. ^{220}NDFYSYDRE motif and ^{110}DDMD motif, and active site pocket are indicated.

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