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Crystallographic analysis of enzymes involved in fosfomycin biosynthesis

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1 Introduction

Fosfomycin is a natural phosphonate antibiotic discovered in the broth from a soil bacterium *Streptomyces fradiae* (Hendlin, *et al.*, 1969) and shows broad-spectrum activities against Gram-positive and Gram-negative bacteria (Allerberger & Klare, 1999; Nakazawa, *et al*., 2003; Cassone, *et al*., 2004). Therefore, fosfomycin is widely used to treat acute cystitis.

Although fosfomycin has a simple structure (Christensen, *et al.,* 1969), the biosynthetic pathway has not been fully elucidated (Seto & Kuzuyama, 1999; Metcalf & van der Donk, 2009). To date, the pathway has been proposed to involve five sequential reactions catalyzed by Fom1, Fom2, FomC, Fom3, and Fom4 enzymes. The initial biosynthetic reactions begin with a conversion of phosphoenolpyruvate (PEP) to phosphonopyruvate. This conversion is catalyzed by PEP phosphomutase, which is an enzyme common to the biosynthesis of all natural phosphonates including the herbicide bialaphos and the antibiotic FR-900098. In fosfomycin biosynthesis, Fom1 catalyzes the first step to form phosphonopyruvate, which is decarboxylated to phosphonoacetaldehyde by phosphonopyruvate decarboxylase (Fom2). Phosphonoacetaldehyde is then reduced to 2-hydroxyethylphosphonate (HEP) by FomC dehydrogenase. The radical-SAM enzyme, Fom3, has been proposed to catalyze methylation of the C2 of HEP to form 2-hydroxypropylphosphonate. Finally, 2-hydroxypropylphosphonate epoxidase (Fom4) converts HPP to fosfomycin.

Curiously, Fom1 has an extra cytidylyltransferase (CyTase) domain at the N-terminus in addition to the C-terminal PEP phosphomutase domain, whereas neither BcpB nor FrbD encoding each PEP phosphomutase for the biosynthesis of bialaphos and FR-900098 has the CyTase domain. Here, to obtain a clue to elucidate the function of CyTase domain, we expressed, purified, crystallized, and obtained preliminary X-ray diffraction data of CyTase domain of Fom1 from a fosfomycin-producing *Streptomyces wedmorensis*.

2 Materials and Methods

Purification

CyTase domain of Fom1 was overexpressed in *E. coli* BL21-Codon-Plus (DE3)-RIL as a host using 0.1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) for induction. From cell lysate prepared by sonication, TK0280 was purified through heat treatment, Ni^{2+}-NTA affinity chromatography and Superdex 200 gel filtration chromatography.

Crystallization

Screening of crystallization condition was initially performed with Crystal screen I and II, Wizard classic I, II, and III, and PEG/ION by hanging drop vapor diffusion method. The crystallization conditions were further optimized by changing pH and concentration of precipitant.

Data collection and processing.

The X-ray diffraction data of native proteins were collected using the beamline, NW12A at PF.
The image sets were integrated and scaled using HKL2000.

3 Results and Discussion

Expression and purification of CyTase domain

Recombinant CyTase domain fused with histidine tag at the N-terminal was expressed in *E. coli* and purified through successive column chromatographies. CyTase domain was purified to homogeneity on SDS-PAGE analysis.

Crystallization and collection of datasets

As a result of crystal screening, we obtained a crystal. We performed optimization the crystallization condition. We are now trying to collect dataset of X-ray diffraction data at PF. Further studies will provide the first structure of the CyTase domain of Fom1 involved in phosphonate biosynthesis and also a clue to understand the mechanism of substrate recognition, which will clearly define the function of the CyTase domain in fosfomycin biosynthesis.

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