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X-ray crystallographic analysis of a ferric ion binding protein (FbpA) from a pathogenic marine bacterium, *Vibrio metschnikovii*

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1 Introduction

Vibrio species is a group of ubiquitous bacteria that inhabits in a wide range of aquatic and marine environments. One of the pathogenic *Vibrio* species, *V. metschnikovii* can be isolated from river water, sewage, and contaminated seafood. Human infected by *V. metschnikovii* will suffer from abdominal pains, diarrhea, dyspnoeic symptoms, weakness, vomiting, nausea, vertigo and headache.

As a Gram-negative bacteria, *V. metschnikovii* has a ferric ion binding protein termed VmFbpA. VmFbpA is responsible for Fe^{3+} binding in its periplasm and delivering Fe^{3+} into the cytoplasm. Therefore, the control of Fe^{3+} binding activity by VmFbpA will be the key to inhibit the growth of *V. metschnikovii*. This project is to analyze the structure of VmFbpA and understand the molecular mechanism of its Fe^{3+} binding and inhibition [1].

2 Experiment

The gene encoding VmFbpA (WP_004394209.1) was cloned into a modified pET-28a vector, and overexpressed in *E. coli* BL21(DE3). Large-scale purification of VmFbpA was performed by affinity chromatography using Ni-nitrilotriacetic acid column and gel filtration chromatography using Superdex200 10/300 GL increase column. The purified protein was then concentrated to ~15 mg/ml for crystallization.

Initial crystallization screening was performed in 96-well Violamo Protein Crystallization Plates (As One) using commercially available kits, namely Crystal Screen HT, Index HT (Hampton Research), and Wizard I and II (Emerald BioSystems) at 293 K. A crystallization drop was prepared by mixing 0.5 µL protein solution and 0.5 µL reservoir solution and was equilibrated against 40 µL reservoir solution. Furthermore, crystallization conditions were optimized by the sitting-drop vapor-diffusion method in 24-well Cryschem Plates (Hampton Research). A crystallization drop was prepared by mixing 1.0 µL protein solution and 1.0 µL reservoir solution and was equilibrated against 500 µL reservoir solution.

Crystals of apo VmFbpA were obtained using a reservoir composition of 0.25 M ammonium tartrate dibasic, 25% PEG3350, 100 mM Tris-HCl (pH 7.0). Each crystal was picked up in a mounting loop and cooled in a cold nitrogen gas stream using 20% (v/v) glycerol as a cryoprotectant. X-ray diffraction experiments for apo VmFbpA crystals were performed on beamline AR-NE3A at Photon Factory. The data were indexed, integrated, and scaled using the XDS program. Initial structural models of apo VmFbpA were obtained by molecular replacement with Morlep using TtFbpA (PDB entry: 3WAE) as the search model. Further model building and refinement were performed with REFMAC5, Coot, and Phenix.

3 Results and Discussion

The diffraction data from the VmFbpA crystals were collected to ~2.0 Å. The crystal structure of VmFbpA was finally solved and deposited into the Protein Data Bank (ID: 7W3W). The structure information was also used in the docking analysis between VmFbpA and rosmarinic acid (RA), a VmFbpA inhibitor from rosemary [1].

The results showed that RA can competitively inhibit the Fe^{3+} binding by docking at the Fe^{3+} binding site of VmFbpA (Fig. 1).

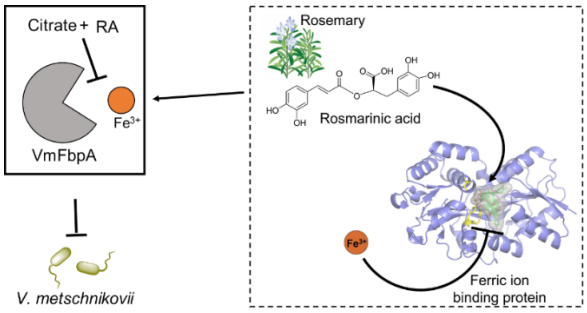

Fig. 1: The growth inhibition of *V. metschnikovii* by the restriction of Fe^{3+} uptake.

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