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X-ray crystal structure of ferric-citrate binding protein FecB

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1 Introduction

Iron is an essential nutrient for bacteria as it plays an essential role in various cellular processes. Many Gram-negative pathogens acquire iron through siderophore-independent systems such as ferric-citrate uptake. FecB is a periplasmic binding protein that captures ferric-citrate in the periplasm and transfers it to the inner membrane transporter complex FecCDE (Fig. 1). In this study, we determined the crystal structures of *V. alginolyticus* FecB bound with citrate and ferric-citrate. The structural studies will provide detailed insights into substrate recognition and the conformational transitions that accompany ligand binding, which laid the structural basis for the development of a novel small-molecule inhibitor.

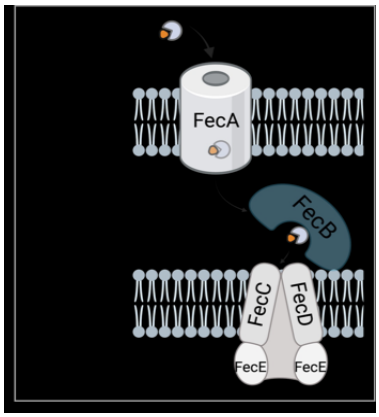

Fig.1: Ferric ion acquisition by FecABCDE

2 Experiment

The FecB-citrate co-crystal was prepared by incubating pure FecB protein with 10-fold sodium citrate overnight. The protein was then subjected to final purification by loading onto a Superdex 200 10/300 GL column using gel filtration buffer (20 mM Tris–HCl pH 8.0 and 150 mM NaCl) and an ÄKTA purifier (GE Healthcare), after concentration then for crystal screening. The co-crystal was obtained at 20°C in 0.1 M HEPES (pH 7.5), 20% w/v polyethylene glycol 10000, and 7 mg/mL of purified FecB-citrate solution.

The FecB-ferric citrate co-crystal was prepared by incubating pure FecB protein with 10-fold ferric citrate overnight. After purifying the protein with Superdex 200 10/300 GL column using gel filtration buffer (20 mM Tris–HCl pH 8.0 and 150 mM NaCl) and an ÄKTA purifier (GE Healthcare), the protein was concentrated for crystal screening. The co-crystal was obtained at 20°C in 0.1 M HEPES (pH 7.5) and 20% w/v polyethylene glycol 10000, with 8.6 mg/mL of the purified FecB-ferric citrate solution.

All the crystals were transferred to a cryoprotectant solution [reservoir solution supplemented with 20 % (v/v) glycerol] and then flash cooled in a stream of nitrogen gas. X-ray diffraction experiments were performed with an X-ray wavelength of 1.00000 Å at beamline AR-NE3A of the Photon Factory (KEK).

3 Results and Discussion

The crystal structure with citrate (PDB: 8Y4V) was solved at 2.3 Å resolution in the space group $P\,2_12_12_1$. The asymmetric unit contained three FecB molecules. While the crystal structure of ferric citrate (PDB: 8Y4Y), was obtained at 2.5 Å resolution in the space group of $I\,422$. Comparing the two structures, there was only one citric acid molecule present in the citrate-bound FecB structure (Fig. 2A), whereas two citric acid molecules and one ferric ion were observed in the binding pocket of the ferric citrate bound FecB structure (Fig. 2B).

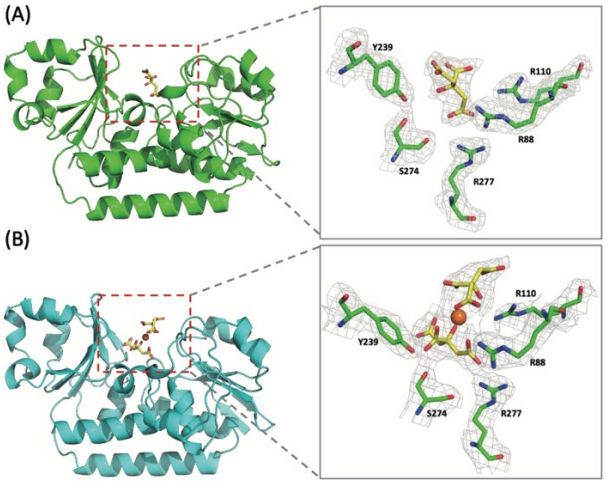

Fig.2: Crystal structure and binding model of citrate(A) and ferric citrate(B) bound to FecB.

FecB-ferric citrate complex includes a ferric ion coordinated by two citric acid molecules. Two tyrosine residues (Tyr-218 and Tyr-239) were present in the binding pocket, whose distances to the bottom citric acid were 5.2 Å and 2.5 Å, respectively. In addition, there were three Arg residues (Arg-88, Arg-110, Arg-277) around the binding site, in which the distances to the bottom citric acid of Arg-88 and Arg-277 were 2.8 and 2.5 Å, respectively, whereas the distances to the top citric acid of Arg-110 was 2.4 Å, respectively. It shows that the FecB protein relies on citrate as a siderophore for ferric ion transport. Ferric ion coordination analysis showed an octahedral geometry,

formed by six ligands contributed from two citric acid molecules (Fig. 3).

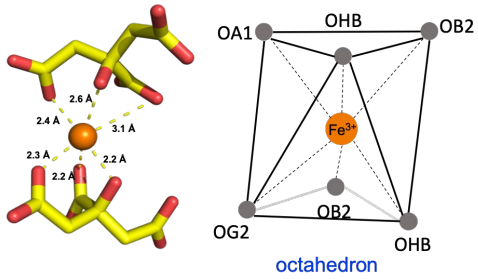

Fig.3: Ferric ion coordination

The crystal structure indicates that FecB acquires ferric ions via a citrate-mediated siderophore mechanism. This finding enriches our understanding of periplasmic binding protein, and provides fundamental insights into their potential as targets for antibacterial drug discovery.

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References

[1] J. Jiang, *et al*., *Angew. Chem. Int. Ed*. **63**, e202411688 (2024)

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