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Molecular recognition of antibacterial porphyrins by IsdH-NEAT3, a protein involved in heme acquisition in pathogenic *Staphylococcus aureus*.

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Introduction

Antibiotic resistance is a serious problem that threatens public health and diminishes our capacity to combat disease [1]. Non-iron metalloporhyrins (porphyrins containing a metal other than iron) have shown antibacterial properties against pathogenic bacteria including *Staphylococcus aureus*. Little is known about the molecular mechanism(s) of action of these compounds, and in particular how they reach the interior of the bacterial cells.

A reasonable hypothesis has suggested that these compounds penetrate the cell employing the same transporters used by the bacteria to steal heme from the host organism. Heme-transport in *S. aureus*, so-called Isd system, is composed of nine proteins distributed in cell wall, membrane, and cytoplasm. Among them, protein IsdH is the first heme receptor in Isd pathway. Previously, we reported the crystal structure of heme-binding domain of IsdH, so-called NEAT3, in both apo (heme-free) and holo (heme-bound) forms [2].

Herein we investigated the structural basis of recognition of two antibacterial non-iron porphyrins by IsdH-NEAT3. Two crystal structures at nominal resolutions of 1.7 Å and 2.7 Å revealed the nature of their interaction.

Experimental

Purified IsdH-NEAT3 was mixed with non-iron porphyrins Ga(III)-PPIX (PPIX, protoporphyrin IX) or Mn(III)-PPIX. Single crystals of protein/porphyrin complexes were grown by vapor diffusion methods. Crystals reached full size after one month. Suitable crystals of each complex were harvested, soaked in a solution with glycerol, and frozen in liquid N_2. Data collection for this project was carried out in beamlines BL-5A, AR-NE3A, and AR-NW12A of the Photon Factory) under cryogenic conditions (100 K). Diffraction images of single-crystals of IsdH-NEAT3 in complex with Ga(III)-PPIX or Mn(III)-PPIX were processed with MOSFLM, and merged and scaled with SCALA of the CCP4 suite. Three-dimensional structures were determined by the method of molecular replacement with PHASER. Models were refined with REFMAC5 and COOT. Coordinates of refined models have been deposited in the RCSB Protein Data Bank under accession codes 3QUG (IsdH-NEAT3/Ga(III)-PPIX complex) and 3QUH (IsdH-NEAT3/Mn(III)-PPIX complex).

Results and Discussion

We determined the crystal structure of IsdH-NEAT3 in complex with Ga(III)-PPIX and with Mn(III)-PPIX at a resolution of 1.7 Å and 2.7 Å, respectively (Fig. 1). Continuous electron-density features between oxygen atom of Tyr642 and metal indicate this bond is essential to capture metalloporphyrin in the binding pocket of receptor. Comparison of these structures with that of Fe(III)-PPIX demonstrated they are indeed nearly indistinguishable from each other: Overall fold, secondary structure elements, stoichiometry, and relative position of the porphyrin ligand were very similar in the three structures. This conclusion is surprising because these three types of crystals were grown in different precipitant solutions and crystallized in different space groups.

Our results revealed mechanistic equivalence among different porphyrins, thus supporting the hypothesis that antibacterial non-iron porphyrins use Isd system to penetrate the bacterial cell. These data highlights the attractive idea of using Isd system as a novel route to deliver antibacterial compounds into *S. aureus*.

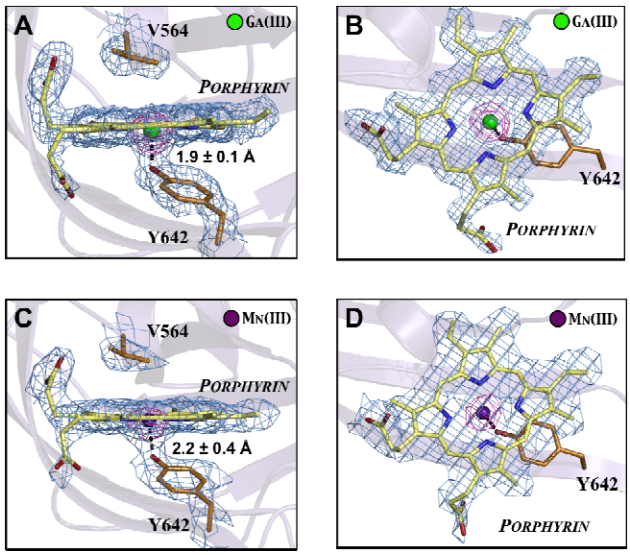

Figure 1. Electron density map of Ga-PPIX (A, B) and Mn-PPIX (C, D) bound to IsdH-NEAT3.

References

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