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Crystal structure of the IsdH linker-NEAT3 involved in heme acquisition in *Staphylococcus aureus*.

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1 Introduction

Pathogenic bacteria are equipped with virulence factors to prosper in the harsh environment of the host, including its defense systems. Iron is an essential nutrient that pathogenic bacteria need in order to grow, and for that, numerous bacteria has developed a system to extract and acquire iron from the host, for example from the heme group contained in the molecule of Hemoglobin (Hb).

The heme-acquisition system in *Staphylococcus aureus* is termed iron surface determinant (Isd). The Isd system is composed of nine proteins, of which IsdH located at the cell-wall facing the extracellular space extract heme from Hb. Inactivating IsdH is a promising antibacterial strategy, but an incomplete knowledge about the mechanism of action is hampering progress.

IsdH is a 101-kDa protein comprising three NEAr-iron Transporter (NEAT) domains connected by linker regions. To understand the mechanism of extraction of iron from human Hb we have determined the crystal structure of the linker-NEAT3 region with heme bound at 1.80 Å [1].

2 Experiment

Full-length IsdH with an N-terminal His_6 tag was cloned in vector pET28b. Recombinant IsdH was expressed in *E. coli* Rosetta2 (DE3) at 37 °C upon induction with 1 mM IPTG after the culture reached an OD of about 0.5 for about four hours. Cells were harvested by centrifugation, resuspended in lysis buffer containing 20 mM imidazole, and disrupted by sonication. The soluble fraction after centrifugation at 40,000 × *g* for 30 min was subjected to immobilized-metal affinity chromatography IMAC in Ni^{2+}-NTA. The protein was eluted with a gradient of imidazole (20 - 500 mM). The fractions containing IsdH were pooled together, and treated with the protease thrombin at 1 U/mg protein overnight, followed by a second IMAC. Only flow-through fractions were collected. Finally, the protein was subjected to size-exclusion chromatography in 50 mM phosphate buffer at pH 7.4. During the purification, the protein fraction obtained did not correspond to the full-length protein, but to the linker-NEAT3 region as determined from the structural data.

The linker-NEAT3 protein obtained from above at 10 mg ml^{-1} was crystallized in a solution composed of 0.2 M potassium chloride and 20% PEG 3350 (Hampton Research) at 298 K. Diffraction data were collected in beamline BL5A at the Photon Factory (Tsukuba) at 100 K. Diffraction images were processed with standard programs MOSFLM and SCALA. Molecular replacement was carried out with PHASER, and refined with REFMAC5 and COOT.

3 Results and Discussion

The structure of linker-NEAT3 with heme bound is presented at 1.8 Å resolution (Fig. 1). The structure included residues Asn476 to Val537 corresponding to the linker, and Thr538 to Asn656 belonging to NEAT3. The linker displays three α-helices (H1, H2, and H3). The NEAT3 domain is mostly made of β-sheet structure and two short helical segments. Tyr642 located within coordinating distance from the metal ion of heme (2.11 ± 0.05 Å).

The linker domain and the NEAT3 domain interact with each other through the heme-binding pocket, but the linker domain does not interact directly with the heme moiety (Fig. 1). The analysis of the linker-NEAT3 interface using the PISA server indicated the presence of hydrogen bonds and salt bridges. When compared to the structure of linker-NEAT3 with no heme bound, it is observed that binding of heme does not cause conformational changes. We also show that the heme moiety undergoes a rotation during transfer from Hb to NEAT3

Collectively, this report contributes to improve our understanding of the heme acquisition mechanism from Hb to IsdH. Since heme-acquisition is a general phenomenon among pathogenic bacteria, these data may help to clarify the mechanism of other Isd surface proteins such as in *Bacillus anthracis*. And because NEAT domains are described to interact with other proteins from the host (*e.g.* fetuin, asialofetuin, fibrinogen, fibronectin, loricrin, involucrin, cytokeratin K10, and vitronectin), these results can be used as a benchmark for further studies aiming at their characterization at the molecular level.

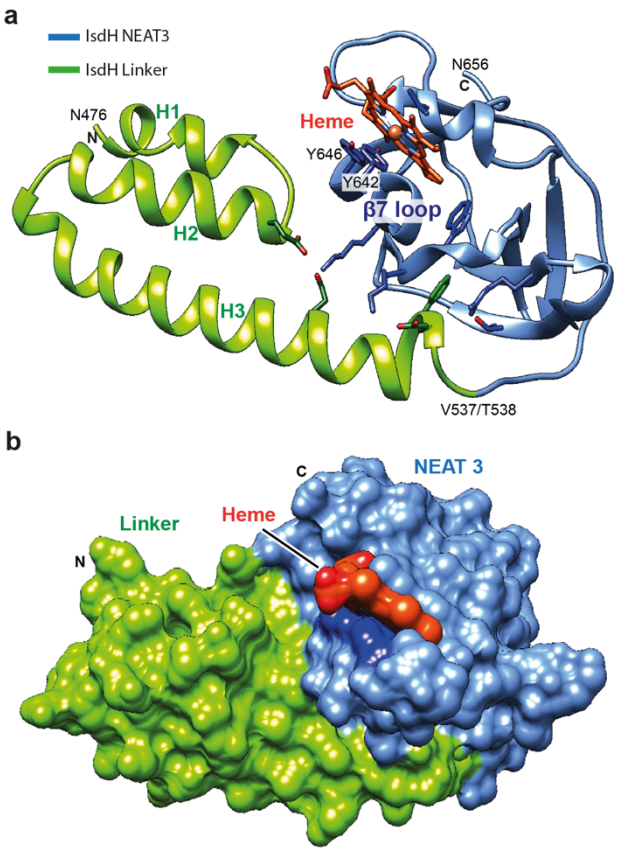

Fig. 1: Crystal structure of IsdH linker-NEAT3 with heme bound. (a) Cartoon model. (b) Surface model. The linker, NEAT3, and heme moieties are depicted in green, blue, and orange, respectively. The figure was adapted [1].

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References

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