5A, 6A, NW12A, NE3A/2010G530, 2008G587, 2007G052, 2011A1893

Structural elucidation for the metal storage function of *Helicobacter pylori* neutrophil-activating protein

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1 Introduction

Helicobacter pylori causes severe diseases such as chronic gastritis, peptic ulcers, and stomach cancers. *H. pylori* neutrophil-activating protein (HP-NAP) promotes adhesion of neutrophils to endothelial cells, and induces the production of reactive oxygen radicals. The crystal structure of HP-NAP from *H. pylori* strain 26695 (HP-NAP 26695) was first determined at 2.5 Å resolution [1]. HP-NAP is a ferritin-like iron storage protein, and is a dodecameric protein consisting of 17 kDa monomers with a central cavity where iron ions bind. HP-NAP can bind up to 500 atoms of iron per dodecamer in vitro. Ferritin binds metals such as Cd^{2+}, Zn^{2+}, Tb^{3+}, or Ca^{2+} in addition to Fe^{2+}. Due to the structural similarity between HP-NAP and ferritin, HP-NAP may bind metals other than irons, although, to our knowledge, there are no such reports. In order to understand the metal-storing function of HP-NAP, we determined the crystal structures of HP-NAP from strain YS39 (HP-NAP YS39) in the apo and metal-bound forms such as Fe^{3+}, Zn^{2+} and Cd^{2+} [2, 3]. HP-NAP YS39 was clinically isolated in Japan, and differs from HP-NAP 26695 at three residues (E46G, V59A and I73L).

2 Experiment

HP-NAP YS39 was expressed in *E. coli*, and purified using nickel affinity chromatography. Crystallization was carried out with the sitting-drop or hanging-drop vapor-diffusion method at 20°C. The protein solution of HP-NAP in 50 mM Tris-HCl and 0.1 M L-arginine (pH 8.8) was mixed with an equal volume of a reservoir solution to form the droplet. Crystals of the apo form were prepared using a reservoir solution containing 20% ethylene glycol. To obtain Zn^{2+}-bound crystals, the crystals were soaked into a solution containing 20% ethylene glycol and 20 mM $ZnSO_4$ for 15 min. To obtain Fe^{3+}-bound crystals, a reservoir solution consisting of 20% ethylene glycol and 10 mM iron(II) sulphate was used. To obtain Cd^{2+}-bound crystals, a reservoir solution containing 50 mM $CdSO_4$, 1.0 M sodium acetate, and 0.1 M HEPES-NaOH (pH 7.5) was used. For all crystallization conditions, crystals of cubic or rectangular form appeared and grew to an approximate size of 0.2 mm on a side.

Crystals were transferred into a solution of 30% glycerol in the mother liquor, and were flash-frozen in a stream of N_2 at 95 K. X-ray diffraction data were collected at beamlines BL5A, BL6A, NW12A, and NE3A, and were processed and scaled with HKL2000. The structures were solved by the molecular replacement method with MOLREP in the CCP4 suite. Crystallographic refinements and structural adjustments were performed with REFMAC5 and COOT, respectively.

3 Results and Discussion

The structures of HP-NAP YS39 were determined at 2.2-2.5 Å resolution. A total of 12 protomers form a dodecamer like a spherical shell about 90 Å in diameter with crystallographic point group 23. The internal cavity of the dodecamer is about 50 Å in diameter. The overall structures of HP-NAP YS39 determined here are similar to that of HP-NAP 26695.

In a Zn^{2+} or Cd^{2+}-bound form, two zinc or two cadmium ions and their bridged water molecule were located at the ferroxidase center (FOC). The two zinc ions are coordinated in a tetrahedral manner to the conserved residues among HP-NAP and Dps proteins. The two cadmium ions are coordinated in a trigonal-bipyramidal and distorted octahedral manner. In both structures, the second ion is more weakly coordinated than the first. In a Fe^{3+}-bound form, however, only one iron ion was located at almost the same position as the first (Zn1 or Cd1). It is possible that the di-iron sites are occupied only by Fe^{2+}, which is rapidly oxidized to Fe^{3+}. Therefore, in crystals of YS39 Fe following long exposure to oxygen, there is no second ion in the FOC. In the FOC of YS39 Zn, two zinc ions are separated by a distance of 3.3 Å, and two cadmium ions are separated by 4.2 Å, longer than the di-zinc distance of YS39 Zn. In YS39 Fe or YS39 Zn, another ferric or zinc ion is found inside of the negatively-charged three-fold-related pore, indicating that the pore is suitable for metal ions to pass through.

Between the apo and metal-bound structures, a major difference was observed. In comparison with the apo form, the side chains of Asp52 and Glu56 move toward the first metal ion (Fe1, Zn1, or Cd1) in metal-bound forms. Conversely, the Trp26 side chain flips out of the FOC. After the conformational change of Trp26, the side chains of Asp52 and Glu56 can be chelated by the first metal ion.

In conclusion, both zinc and cadmium ions can bind to the FOC, indicating that HP-NAP can store zinc and cadmium ions in addition to iron ions.

References

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