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Structural study for the neutrophil-activating protein from *Helicobacter pylori*

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Introduction

Helicobacter pylori, a Gram-negative bacterium, chronically infects up to 50% of the world's human population, and causes severe diseases such as chronic gastritis, peptic ulcers, and stomach cancers. *H. pylori*-induced gastritis is typically associated with infiltration of the infected stomach mucosa by neutrophils. *H. pylori* neutrophil-activating protein (HP-NAP) promotes adhesion of neutrophils to endothelial cells, and induces the production of reactive oxygen radicals. HP-NAP is a major antigen in the human immune response to *H. pylori* infection.

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 [2]. 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 tried to determine the crystal structures of HP-NAPs in the apo form and in the metal-bound forms such as Cd^{2+} and Zn^{2+}.

Materials and Methods

We used HP-NAPs from the *H. pylori* YS29 and YS39 strains (HP-NAP YS29 and YS39) clinically isolated in Japan. The amino-acid sequences of HP-NAP YS29 and YS39 are more than 97% identical to that of HP-NAP 26695. HP-NAPs were expressed in *E. coli*, and purified using nickel affinity chromatography. L-arginine was applied to a protein solution during the purification and crystallization in order to suppress aggregation of proteins. 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 9.3) was mixed with an equal volume of a reservoir solution to form the droplet. Crystals of the apo form of HP-NAP YS29 were prepared using a reservoir solution containing 2.0 M ammonium sulfate and 0.1 M Tris-HCl (pH 7.5). In order to determine the metal-bound forms such as Cd^{2+} and Zn^{2+}, co-crystallization and soaking experiments were attempted. Crystals of the Cd^{2+}-bound form of HP-NAP YS29 were prepared using a reservoir solution containing 50 mM $CdSO_4$, 1.0 M sodium acetate, and 0.1 M HEPES-NaOH (pH 7.5). Crystals of the apo form of HP-NAP YS39 were prepared using a reservoir solution containing 25% Ethylene glycol. And the crystals were soaked into the reservoir solution containing 20 mM $ZnSO_4$. For all crystallization conditions, crystals of cubic or rectangular form appeared and grew to an approximate size of 0.2 mm on a side.

X-ray diffraction data from flash-frozen crystals were collected at beamlines BL5A, NW12A, and NE3A, and were processed and scaled with HKL2000. The structures were solved by the molecular replacement method with the program MOLREP in the CCP4 suite. The structure of the chain A of the HP-NAP 26695 (Protein Data Bank code 1JI4) was used as a search model. The initial model was subjected to rigid-body refinement, and then subjected to crystallographic refinement with the program REFMAC5 in the CCP4 suite, followed by manual model fitting with the program COOT. After iterative cycles of model fitting and refinement, metal ions were added to peaks of the F_o-F_c electron density greater than 10 σ.

Results and Discussion

The structures of HP-NAP YS29 and YS39 were determined at 2.1-2.5 Å resolution higher than that of the previously determined structure of HP-NAP 26695. The crystals of HP-NAP YS29 and YS39 belong to cubic space group $F432$, whereas the crystal of HP-NAP 26695 belongs to monoclinic space group $P2_1$. The V_M value is 3.6 $Å^3$/Da, and the solvent content is 66%. The relatively high solvent content is probably due to the large internal cavity. HP-NAP YS29 and YS39 contain one monomer in the asymmetric unit. Each monomer is related by crystallographic two-fold and three-fold axes, and then forms a dodecamer displaying 23 symmetry using symmetry-related molecules. Overall structures of HP-NAP YS29 and YS39 are very similar to that of HP-NAP 26695. Although the HP-NAP 26695 contains a ferric iron at the ferroxidase center, the HP-NAP YS29 in the Cd^{2+}-bound form contains two cadmium ions and their bridged water molecule at the ferroxidase center. By comparing the apo and Cd^{2+}-bound forms of HP-NAP YS29, binding of cadmium ions causes the structural change of the side chains in the vicinity of the ferroxidase center (manuscript in preparation).

Refinement of the Zn^{2+}-bound structure of HP-NAP YS39 is now in progress.

References

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