

Crystal structure of hemin binding protein 35 kDa (HBP35) from *Porphyromonas gingivalis*

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Introduction

A variety of cell envelope hemin binding proteins in *P. gingivalis* have been reported. It has been suggested that hemin play an essential role in the growth and virulence of *P. gingivalis*, and that the hemin binding is the first step in the uptake of these molecules to utilize for their own living. Hemin binding protein 35-kDa (HBP35) possesses a conserved motif (WCGxCx) present at the active center of many thioredoxins, and suggested the heme-binding is related the Cys-residues in thioredoxin motif. HBP35 combine the hemin, but has no ability to bind the hemoglobin molecules. In addition, HBP35 selects the specific metal ions such as iron into cell for its living. Furthermore, HBP35 involves the expression of virulence of *P. gingivalis* hemin uptake system. However, the fundamental factor of heme binding and uptake in *P. gingivalis* by HBP35 is unknown.

In the study, we report the crystallization and X-ray crystallographic study of HBP35, aiming to solve the structure and gain insights into the functional aspects of this protein.

Experiments and Results

HBP35 was expressed in E.coli JM109 grown in LB medium, and purified by Q-Sepharose, Butyl HP column and gel-filtration column. The first crystallization screening was performed using large-scale protein-crystallization robot (PXS). A few crystals were obtained using polyethylene glycol as a precipitant. After optimization of this condition, crystals with dimensions of 0.05×0.05×1 mm were formed by the conventional vapour-diffusion method. For the multi-wavelength anomalous diffraction (MAD) method, selenomethionyl protein crystals of HBP35 were prepared in the same conditions.

Diffraction images were collected on a charge-couples device detector at the NW12 beamline of the Photon Factory, under cryogenic condition (100 K) using flash cooling technique. Crystal was soaked into the crystallization buffer contained 30% PEG400. The dataset up to a resolution of 1.8 Å was collected and processed using HKL2000 software package. The crystal belongs to the space group $P2_12_12_1$ with unit cell parameters $a=49.0$ Å, $b=58.3$ Å, $c=114.4$ Å. Nine out of ten selenium sites in the asymmetric unit were located with SOLVE. The

phases were improved with RESOLVE. Tracing the electron density and the model building for the HBP35 molecule was carried out automatically using ARP/wARP.

The final model refined to 30 - 1.8 Å resolution has an R -factor of 17.6% and an R_{free} of 21.1%. HBP35 comprises two structural domains, which are an N-terminal domain (31-140) and C-terminal domain (141-344). N-terminal domain consists of thioredoxin domain and β -sandwich domain. The availability of three-dimensional structures of this protein allows us to identify regions that are important to hemin binding and hemagglutinin activity.



Figure1 structure of HBP35

References

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