BL17A/2013G152

Crystal structure analyses of dipeptidyl peptidase 11 from *Porphyromonas gingivalis*

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1 Introduction

Periodontitis is a bacterially induced inflammatory disease that destroys the periodontal tissues, eventually leading to tooth loss. Periodontitis is widely regarded as the second most common disease worldwide, and chronic periodontitis affects approximately 750 million people as of 2010. *Porphyromonas gingivalis*, a Gram-negative, anaerobic bacterium, is a major pathogen associated with the chronic form of periodontitis. Because *P. gingivalis* is an asaccharolytic bacterium that gains its metabolic energy by fermenting amino acids, peptidases of *P. gingivalis* that provide di- and tripeptides are essential for the metabolism of the bacterium, and much attention has been paid to dipeptidyl peptidases (DPPs) from *P. gingivalis*. Recently, novel DPPs, DPP5 (PgDPP5), DPP7 (PgDPP7) and DPP11 (PgDPP11), have been identified from *P. gingivalis*. *P. gingivalis* peptidases PgPTPA, PgDPP4, and PgDPP5 have been classified as clan SC, family S9 in the MEROPS database, while PgDPP7 and PgDPP11 have been assigned to another type of serine peptidase family, S46 in clan PA. Whereas PgDPP7 exhibits a broad substrate specificity for both aliphatic and aromatic residues at the P1 position (NH_2-P2-P1-P1'-P2'-…, where the P1-P1' bond is the scissile bond), PgDPP11 exhibits a strict substrate specificity for acidic residues (Asp/Glu) at the P1 position. Because Asx (Asp and Asn) and Glx (Glu and Gln) are the most abundantly utilized amino acids in this bacterium, PgDPP11 plays a critical role in the metabolism of *P. gingivalis* by degrading polypeptides carrying Asp and Glu.

2 Experiment

PgDPP11 was expressed and purified as described elsewhere [1]. A synthetic gene coding for full-length PgDPP11 (residues 1-720), codon-optimized for expression in *E. coli*, was purchased from Genscript (Piscataway, USA) and cloned into the pET22b expression plasmid (Merck, Darmstadt, Germany). The mature PgDPP11 was composed of 699 amino acids (residues 22 to 720), with a theoretical molecular weight of 79618.27 and an isoelectric point of 5.87. Se-Met-substituted PgDPP11 was expressed using the Overnight Express Autoinduction System 2 (Merck). The Se-Met derivative was purified in a manner similar to that used for wild-type PgDPP11 [2].

To obtain peptide-free PgDPP11 crystals, the samples were crystallized using the hanging-drop method, in which 1 µl of protein solution (10 mg/ml PgDPP11 in 80 mM Tris-HCl, pH 8.5) was mixed with the same volume of reservoir solution (20 % (v/v) glycerol, 16 %(m/v) PEG8000 and 0.16 M tri-potassium citrate) and incubated at 293 K. The drops were suspended over 200 µl of reservoir solution in 48-well plates [1]. Peptide-free crystals were also obtained using a counter-diffusion crystallization method under a microgravity environment in the Japanese Experimental Module "Kibo" at the International Space Station (ISS) [2].

Diffraction data were collected by the rotation method at 100 K using an ADSC Quantum CCD detector with synchrotron radiation source at the Photon Factory BL17A. Initial phases were determined for the peptide-free Se-Met PgDPP11 using the multi-wavelength anomalous diffraction (MAD) method. The Se-Met-substituted PgDPP11 was refined at 2.5 Å resolution, and the resulting model was used as the starting model for the structural refinement of wild-type PgDPP11.

3 Results and Discussion

The crystal structure of PgDPP11 was determined using MAD method at 2.5 Å resolution by analyzing a Se-Met substituted PgDPP11 crystal. The final model was obtained from a native data set using a space-grown crystal at 1.66 Å resolution. A protomer of PgDPP11 is situated in the asymmetric unit. Two protomers of PgDPP11 are related by a crystallographic twofold axis of the $C222_1$ crystal and form a dimer. Each subunit contains a catalytic double β-barrel domain harboring the Asp-His-Ser catalytic triad and an α-helical domain that caps the active site. The crystal structure analyses clearly explain the molecular basis of the Asp/Glu specificity of PgDPP11, which is determined by Arg673 in the S1 subsite. The present structural analyses will support the design of specific inhibitors of DPP11s produced by pathogenic organisms.

References

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