Crystal Structure of Dipeptidyl Peptidase 11 from *Porphyromonas gingivalis*: the Molecular Basis of Substrate Specificity among the Family S46 Peptidases

Porphyromonas gingivalis is a major pathogen associated with the chronic form of periodontitis. Dipeptidyl peptidase 11 belongs to the family S46 of serine peptidases and preferentially cleaves substrates with Asp/Glu at the P1 position. Crystal structure analyses of DPP11 from *P. gingivalis* (PgDPP11) revealed that the positively-charged side chain of Arg673 in the S1 subsite is essential for recognition of the negatively-charged Asp/Glu side chain at the P1 position of the bound substrate. The present structural analyses could be useful templates for the design of specific inhibitors of DPP11s from pathogenic organisms.

Periodontitis is a bacterially-induced inflammatory disease that destroys the periodontal tissues, eventually leading to tooth loss. *Porphyromonas gingivalis* 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*. The dipeptidyl peptidase 11 from *P. gingivalis* (PgDPP11) belongs to the family S46 of serine peptidases and preferentially cleaves substrates with Asp/Glu at the P1 position (NH_2-P2-P1-P1'-P2'-…, where the P1-P1' bond is the scissile bond). The molecular mechanism underlying the substrate specificity of PgDPP11 is unknown. In this study, we determined the crystal structure of PgDPP11 and the crystal structure analyses clearly explain the molecular basis of the Asp/Glu specificity of PgDPP11, which is determined by the conserved Arg residue in the S1 subsite [1].

Wild-type and Se-Met substituted PgDPP11 was expressed and purified as described elsewhere [1]. The purified PgDPP11 was crystallized using the hanging-drop method. Crystals of PgDPP11 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). Diffraction data were collected by the rotation method at 100 K using an ADSC Quantum CCD detector at BL-17A. The crystal structure of PgDPP11 was determined using the multi-wavelength anomalous diffraction 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. The final R and R_{free} values were 0.188 and 0.226, respectively, at 1.66 Å resolution. A protomer of PgDPP11 was situated in the asymmetric unit. Two protomers of PgDPP11 were related by a crystallographic twofold axis of the $C222_1$ crystal and formed a dimer (**Fig. 1, left**). Each subunit contains a catalytic double β-barrel domain harboring the Asp227-His85-Ser655 catalytic triad and an

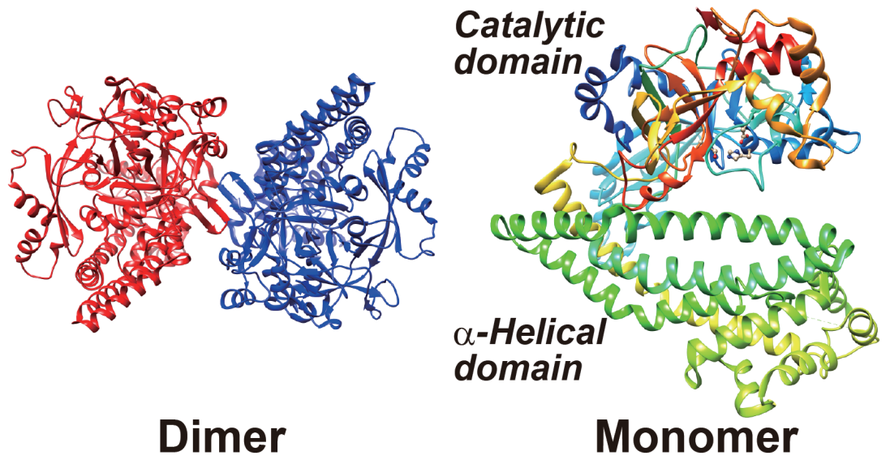

Figure 1: Overall structure of PgDPP11.

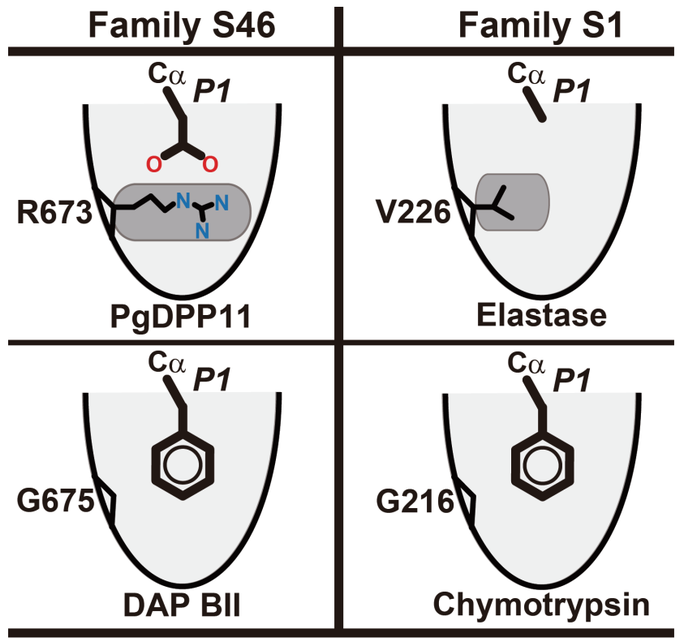

Figure 2: Molecular basis for the substrate specificities of clan PA peptidases.

α-helical domain that caps the active site and is necessary for exopeptidase activity (**Fig. 1, right**). Arg673 in PgDPP11, a crucial residue for the Asp/Glu specificity of PgDPP11, is located in the wall of the S1 subsite. *In silico* docking of a Leu-Asp dipeptide into the active site of PgDPP11 suggested particular interactions between the bound dipeptide and the S1 subsite of PgDPP11. To test the role of Arg673 in substrate binding by PgDPP11, we replaced the Arg residue with alanine and tested the enzymatic activity of the mutant protein on synthetic substrates with Asp/Glu at the P1 position. The mutation resulted in complete loss of activity. This result suggests that Arg673 in PgDPP11 is responsible for the recognition of the Asp/Glu residue at the P1 position of the substrate peptide. Thus, the crystal structure analyses, *in silico* docking studies, and site-directed mutagenesis studies clearly explain the molecular basis of the Asp/Glu specificity of PgDPP11, which is determined by the conserved Arg residue (Arg673) in the S1 subsite.

It is interesting to note that Arg673 in PgDPP11 is structurally equivalent to Val216 in elastase, a clan PA family S1 endopeptidase specific for small, uncharged residues. These residues infill the S1 subsite of each enzyme and prevent the access of longer or bulky side chains at the P1 position of the substrate peptide (**Fig. 2, upper panels**). In contrast, the corresponding positions are occupied by Gly in DAP BII (another enzyme in family S46) and chymotrypsin (family S1), which can accommodate longer or bulky side chains at the P1 position of the substrate peptide (**Fig. 2, lower panels**). Thus, the family S46 exopeptidases and the family S1 endopeptidases adopt structurally equivalent mechanisms in which the side chains located at the wall of the S1 subsite regulate the depth of their S1 subsite, although the peptide digestion patterns are quite different between these peptidase families: "exo" for S46 and "endo" for S1.

REFERENCE

[1] Y. Sakamoto, Y. Suzuki, I. Iizuka, C. Tateoka, S. Roppongi, M. Fujimoto, K. Inaka, H. Tanaka, M. Yamada, K. Ohta, H. Gouda, T. Nonaka, W. Ogasawara and N. Tanaka, *Sci. Rep.* **5**, 11151 (2015).

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