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論　文　内　容　の　要　旨
Abstract of Dissertation

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Antimicrobial resistance is a serious worldwide problem. Moreover, as bacteria are able to circumvent the effects of all the currently available antibiotics, there is an urgent need for the development of new antibiotics with novel modes of action. However, the development of new antimicrobial agents is declining worldwide. Among bacterial pathogens, non-fermenting Gram-negative bacteria (NFGNB) are the main focus of the antimicrobial resistance epidemic. Family S46 peptidases are serine proteases showing dipeptidyl peptidase activity and are distributed in NFGNB (such as Bacteroidetes and Proteobacteria), but are not found in mammals. Since S46 peptidases are important enzymes for the growth of bacteria and distributed in NFGNB, these peptidases could be a novel molecular target of antibiotics for NFGNB. Therefore, the final goal of this study was to find antimicrobials for NFGNB that target S46 peptidase. In this thesis, we worked on the following two studies:

In the first study, we elucidated the substrate recognition mechanisms at the S2 subsite in family-S46 peptidases. While the molecular mechanisms underlining strict specificity at the S1 subsite of S46 peptidases have been well studied, those of relatively broad preference at the S2 subsite of these peptidases are unknown. Here, we evaluated the P2-residue preference of DPP7 from *Stenotrophomonas maltophilia* (SmDPP7) and determined the crystal structures of SmDPP7 in complexes with four kinds of dipeptides (Val-Tyr, Phe-Tyr, Tyr-Tyr, and Asn-Tyr) at resolutions of 2.03 to 1.86 Å. Biochemical studies showed that SmDPP7 prefers hydrophobic amino acids at the S2 subsite in general, except for asparagine. Crystal structure analysis and isothermal titration calorimetry analysis of the Asn-Tyr bindings with SmDPP7 revealed that the exceptionally strong preference for asparagine residue is facilitated by a water-mediated hydrogen bond network in the S2 subsite. An extensive amino acid sequence comparison also revealed that residues in the S2 subsite of S46 peptidases are better conserved than those in the S1 subsite. These observations provide novel insights into the design of a universal inhibitor of S46 peptidases.

In the second study, we investigated an ultra-high-throughput detection method for family-S46 peptidase. Droplet-based microfluidic systems are a powerful tool for biological assays with high throughput. However, because of the oil surrounding the nanoliter and picoliter volumes of droplets, the availability of suitable substrates is limited. we developed new substrates detecting S46 peptidases for establishing an ultra-high-throughput screening method for S46 peptidase-specific inhibitors using a droplet-based microfluidic system. We synthesized a dipeptidyl 7-aminocoumarin-4-acetic acid (ACA) substrate in which the carboxyl group of the dipeptide forms a peptide bond with the amino group at position 7 of ACA and demonstrated the availability of an ACA substrate to detect/screen for DPP activities, S46 peptidases. ACA was retained in the WODL for more than seven days, and the dipeptidyl ACA substrate detected DPP activity of the bacterial

cell in the WODL in addition to detecting purified DPP activity. Thus, the constructed system made the inhibitor (antimicrobial candidate) screening based on S46 peptidase activity by ultra-throughput screening using droplet-based microfluidics passible.