学 位 論 文 内 容 の 要 旨
Abstract of Doctoral Dissertation

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Lipopolysaccharide-bound structure of the antimicrobial peptide cecropin P1 determined by nuclear magnetic resonance spectroscopy
（NMR 法による抗菌ペプチドセクロピン P1 の LPS 結合構造の解析）

Antimicrobial peptides (AMPs) are components of the innate immune system. AMPs may be potential alternatives to conventional antibiotics because they exhibit broad-spectrum antimicrobial activity activities against several organisms, such as Gram-negative and Gram-positive bacteria, viruses, and fungi.

The AMP cecropin P1 (CP1), isolated from nematodes found in the stomachs of pigs, is known to exhibit antimicrobial activity against Gram-negative bacteria with reduced activity against Gram-positive bacteria. In general, CP1 is believed to disrupt the inner membrane through the so-called ‘carpet mechanism’, allowing it to function while not entering the hydrocarbon core of the membrane. However, although CP1 is known to interact with the outer membrane of Gram-negative bacteria, the mechanisms and properties of these functions are not yet fully understood.

I investigated the interaction between CP1 and lipopolysaccharide (LPS) because LPS is the main component of the outer membrane of Gram-negative bacteria and AMPs first encounter and bind to negatively charged LPS. Therefore, elucidation of the detailed structures of AMPs bound to LPS will provide important information about the association between antimicrobial activity and the tertiary structure of the AMP.

In the present study, I examined the LPS-bound state of CP1 using circular dichroism (CD) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy and evaluated its antimicrobial activity by minimum bactericidal concentration (MBC) measurements. CD results showed that CP1 formed an α-helical structure in a solution containing LPS. For NMR experiments, I expressed ^{15}N- and ^{13}C-labeled CP1 in bacterial cells and successfully assigned almost all backbone and side-chain proton resonance peaks of CP1 in water for transferred nuclear Overhauser effect (Tr-NOE) experiments in LPS. I performed ^{15}N-edited and ^{13}C-edited Tr-NOE spectroscopy (Tr-NOESY) for CP1 bound to LPS. Tr-NOE peaks were observed at the only C-terminal region of CP1 in LPS. The results of structure calculation indicated that the C-terminal region (Lys15–Gly29) formed the well-defined α-helical structure in LPS. LPS-bound CP1 had a C-terminal α-helical structure including some positively charged residues (Lys15, Lys16, and Arg17) and hydrophobic residues (Ile18, Ile22, Ala23, Ile24, Ala25, and Ile26). The docking study revealed that CP1 was oriented parallel to the long axis of the lipid A portion of LPS. Especially, Lys15/Lys16 interacted with phosphate at GlcN I via an electrostatic interaction and that Ile22/Ile26 was in close proximity with the acyl chain of lipid A. One dimensional ^{31}P NMR

measurement also showed the peak intensity of the ^{31}P resonance of LPS was significantly changed by binding to CP1. I determined the antimicrobial activity of CP1 and CP1 analogs lacking the C-terminal amino acid residues ($CP1_{1-25}$, and $CP1_{1-20}$) using *E. coli* ML35. The result indicated that the α-helical C-terminal region contributed to the antimicrobial activity of CP1 against Gram-negative bacteria.

The results of this study showed that the C-terminal α-helical structure of CP1 played a crucial role in the recognition of LPS. I anticipate that these findings will be useful for studies of LPS recognition by AMPs. This improved understanding of the molecular basis of AMP activity may assist in the future design of more specific and potent antibacterial agents.