学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Antimicrobial Properties and Mode of Action of Cryptdin-4, a Mouse α-Defensin Regulated by Peptide Redox Structures and Bacterial Cultivation Conditions
（マウス由来αディフェンシン Cryptdin-4 の酸化還元型構造と好気嫌気培養により制御される抗菌活性とメカニズム）

Antimicrobial peptides (AMPs), a diverse family of short peptides that are important for plants and animals, are powerful weapons in host defense mechanisms and are present in all life domains. Defensins, which are widely generated by fungi, insects, and vertebrates, are endogenous cationic AMPs that act as the main effectors of the innate immune system, owing to their broad-spectrum antimicrobial activities. α-Defensins in the mammalian intestinal tract are expressed in the granules of Paneth cells, secreted into the lumen of the small intestinal crypts, and are called cryptdins (crps) in mice. Among cryptdin family members, cryptdin-4 (crp4) has the highest microbicidal activity and contributes to innate immunity in the mouse intestine. Structurally, crp4, with a molecular weight of approximately 4.5 kDa, consists of a three-stranded-sheet structure formed with the aid of three paired disulfide bonds, namely, Cys 1–Cys 6, Cys 2–Cys 4, and Cys 3–Cys 5. In addition to the oxidized form of crp4 (crp4oxi), the disulfide-null reduced-form crp4 (crp4red) has been observed in the reducing environment of the intestinal tract and its physiological role is attracting attention. Crp4 was found to show selective bactericidal activity against intestinal microbiota, which is dependent on disulfide bonds. Crp4oxi and crp4red have also been detected in feces and are associated with homeostasis and dysbiosis of the intestinal microbiota. Therefore, the antimicrobial activity of crp4s in the entire intestinal environment, including the colon and small intestine, has attracted attention. However, the antibacterial mechanism contributing to the bacteria-killing selectivity is poorly understood.

Unfortunately, most existing reports on crp4 focus on the assessment of antibacterial activities against pathogenic bacteria *in vitro*, which have been mainly tested under standard experimental conditions that do not necessarily reflect local conditions in vivo. Within the small intestine, the oxygen concentration decreases in the order of the duodenum, jejunum, and ileum, and becomes highly

anaerobic in the colon. Even in the same region of the intestine, there is a steep oxygen gradient, from high oxygen concentrations near the epithelial surface to severe anaerobic conditions in the center of the lumen. With this background, it is very important to investigate the mechanism of action of oxidized and reduced forms of crp4 (crp4oxi and crp4red) against the same bacteria under aerobic and anaerobic growth conditions, as much remains unknown about their mechanism of action. Therefore, this study compared the antimicrobial activity of crp4oxi and crp4red and their mode of action (MOA) under aerobic and anaerobic culture conditions using *E. coli*, which is a facultatively-anaerobic commensal bacteria of the colon.

Three kinds of MOA of crp4s under different cultivation conditions were confirmed in these experiments: membrane damage; the induction of oxidative stress with ROS production, and the DNA binding action. Fluorescent dye studies revealed that both crp4oxi and crp4red exhibited antimicrobial activity against cells cultured under aerobic conditions via rapid membrane depolarization. Furthermore, the antioxidant treatment experiments suggested that only crp4oxi exhibited antimicrobial activity by the induction and accumulation of reactive oxygen species (ROS). However, under anaerobic culture conditions, the ability of both forms to disrupt the function of bacterial membranes decreased, and activity was greatly reduced, but crp4red maintained some antimicrobial activity. This activity may be due to the inhibition of intracellular functions by DNA binding. Altogether, these data indicate that, according to its redox structure and the environmental redox conditions, crp4 could perform different antimicrobial activities via different mechanisms. It cannot be ruled out that the difference in the efficiency of cellular uptake by crp4red and crp4oxi through membrane permeation and the difference in their DNA-binding capacities may work synergistically and contribute to the extremely large difference in the antimicrobial activity of the two under anaerobic conditions. Finally, suggested by structure change in membrane mimetic conditions by CD data, differences in the structural properties of crp4oxi and crp4red, may be related to differences in the strength of their antimicrobial activities via these mechanisms.

This study suggests that different antimicrobial activities and mechanisms depend on the redox-dependent structure of crp4 and the growth conditions of the target bacteria. This complexity is likely to contribute to the success and stability of this highly conserved host defense system in the constant fight against microbes.