The bactericidal mechanism of lactoferricin B and its fragment revealed by the single GUV method

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学 位 論 文 要 旨

Abstract of Doctoral Thesis

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論文題目：

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論文要旨：

Abstract：

Lactoferricin B (LfcinB) is a peptide fragment produced by hydrolysis of bovine lactoferrin. LfcinB has a strong antimicrobial activity of unknown mechanism. LfcinB is composed 25 amino acids with a highly positively charged peptide, containing five Arg residues and three Lys residues. On other hand, one of shorter fragment of LfcinB (i.e., LfcinB (4-9)) has also have antimicrobial activity. LfcinB (4-9) is composed six amino acids, containing three Arg residues. The interaction of LfcinB and LfcinB (4-9) have been investigated using a suspension of large unilamellar vesicles (LUVs). However, in the LUV suspension method, average value of physical properties can be measured and hence elementary process of phenomena cannot be revealed. In contrast, the single giant Unilamellar vesicle (GUVs) method can be provide detailed information on elementary processes of phenomena such as their rate constants. In this thesis, to elucidate the mechanism of bactericidal activity of LfcinB and LfcinB (4-9), I investigated the interactions of these peptides with lipid membranes using the single GUV method.

<Chapter 2 > To elucidate the mechanistic basis of LfcinB bactericidal activity, I investigated the interaction of LfcinB with *Escherichia coli* and liposomes of lipid membranes. LfcinB induced the influx of a membrane-impermeant fluorescent probe, SYTOX green, from the outside of *E. coli* into its cytoplasm. LfcinB induced gradual leakage of calcein from LUVs of dioleoylphosphatidylglycerol (DOPG) /dioleoylphosphatidylcholine (DOPC) membranes. To clarify the cause of LfcinB-induced leakage of calcein from the LUVs, I used the single GUV method to investigate the interaction of LfcinB with calcein-containing DOPG/DOPC-GUVs. I observed that a

rapid leakage of calcein from a GUV started stochastically; statistical analysis provided a rate constant for LfcinB-induced pore formation, k_p. On the other hand, phase-contrast microscopic images revealed that LfcinB induced a rapid leakage of sucrose from the single GUVs with concomitant appearance of a spherical GUV of smaller diameter. Due to the very fast leakage, and at the present time resolution of the experiments (33 ms), I could not follow the evolution of pore, nor the process of the structural changes of the GUV. Here the word of "local rupture" was used to express the rapid leakage of sucrose and the rate constant of local rupture, $k_{L,}$ was determined. Based on the comparison between k_p and k_L, I concluded that the leakage of calcein from single GUVs occurred as a result of a local rupture in the GUVs, and that smaller pores inducing leakage of calcein were not formed before the local rupture. The results of the effect of the surface charge density of lipid membranes and that of salt concentration in buffer on k_p clearly show that k_p increases with an increase in the extent of electrostatic interactions due to the surface charges. Analysis of LfcinB-induced shape changes indicated that the binding of LfcinB increased the area of the outer monolayer of GUVs. These results indicate that LfcinB-induced damage of the plasma membrane of *E. coli* with its concomitant rapid leakage of internal contents is a key factor for the bactericidal activity of LfcinB.

<Chapter 3> To elucidate the mechanism of bactericidal activity of LfcinB (4-9) with the sequence of RRWQWR, I examined the interaction of LfcinB (4-9) with *E. coli* and single GUVs. First the interaction of LfcinB (4-9) with *E. coli*. was investigated. LfcinB (4-9) did not induce an influx of a membrane-impermeant fluorescent probe, SYTOX green, from the outside of *E. coli* into its cytoplasm, indicating that no damage occurred in its plasma membrane. To examine the activity of LfcinB (4-9) to enter cytoplasm of *E. coli*, I observed the interaction of lissamine rhodamine B red-labeled LfcinB (4-9) (Rh-LfcinB (4-9)) with a single cell of *E. coli* containing a fluorescent probe, calcein, using confocal microscopy. Rh-LfcinB (4-9) entered the cytoplasm without leakage of calcein. To examine the activity of Rh-LfcinB (4-9) to translocate across lipid bilayers, I also investigated the interactions of Rh-LfcinB (4-9) with DOPC/DOPG (1/1)-GUVs using the single GUV method. The results indicate that Rh-LfcinB (4-9) outside the GUV translocated through the GUV membrane and entered its lumen without pore formation. From the analysis of kinetics of the entry of Rh-LfcinB (4-9) into the GUVs, I obtained the rate constants of binding of Rh-LfcinB (4-9) to the GUV membrane and of unbinding from the membrane. Therefore, it can be reasonably considered that Rh-LfcinB (4-9) can translocate across lipid membrane regions of the plasma membrane of *E. coli* to enter its cytoplasm. These results indicate that the target of LfcinB (4-9) for its bactericidal activity is not plasma membrane of *E. coli* but DNA or other proteins inside its cytoplasm.