Effect of membrane potential on action of antimicrobial peptides lactoferricin B and its fragment

（課程博士・様式７）（Doctoral qualification by coursework,Form 7）

学 位 論 文 要 旨

Abstract of Doctoral Thesis

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論文題目：

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Abstract：

Antimicrobial peptides (AMPs) can kill or inhibit growth of bacteria. Several studies suggested the membrane potential affects the activity of AMPs against bacterial cells. However, recent experimental results indicate that membrane potential greatly affects the plasma membrane of bacterial cells such as localization of many proteins and lipids. Thus, the role of membrane potential in the AMP actions becomes unclear. In this thesis, I investigated the role of membrane potential on AMP actions using giant unilamellar vesicles (GUVs) of lipid bilayers. For this purpose, first I developed the method of application of membrane potential to GUVs. Then, I investigated the effect of membrane potential on action of two AMPs using the single GUV method; one AMP is lactoferricin B (LfcinB) derived from bovine lactoferrin and the other is its fragment, LfB4-9 (RRWQWR). These AMPs have different mode of actions; LfcinB induces damage of the plasma membrane which causes rapid permeabilization whereas LfB4-9 can translocate across lipid bilayer without damaging the membrane. I also examined the effect of membrane potential on the interaction of these AMPs with single live *Escherichia coli* cells and single *E. coli* spheroplasts. Based on the obtained results, I discuss the role of membrane potential on the activity of AMPs with lipid bilayers and plasma membranes of bacterial cells.

(Chapter 2) In this chapter, I examined the effect of membrane potential on the action of LfcinB. First, I investigated the interaction of LfcinB with single live *E. coli* cells using confocal laser scanning microscopy (CLSM). *E. coli* cells was loaded with a fluorescent probe, calcein. I found that LfcinB induced rapid leakage of calcein from single *E. coli* cells. To examine the direct interaction of LfcinB with the plasma membrane, I investigated the interaction of LfcinB with single *E. coli* spheroplasts loaded with calcein. This experiment showed that LfcinB induced rapid leakage of calcein from single spheroplasts. These results indicate that LfcinB induced rapid permeabilization due to damage or pore

formation to the plasma membrane of *E. coli* cells. To understand the role of membrane potential, I examined the effect of a proton ionophore, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) on LfcinB-induced leakage from single *E. coli* cells and spheroplasts. I found that presence of CCCP suppressed this leakage. Next, I examined the interaction of Lfcin B with single GUVs of *E. coli*-lipids (i.e., phosphatidylethanolamine/phosphatidylglycerol/cardiolipin (67/23/10 (weight % ratio)) containing a fluorescent probe, AlexaFluor 647 hydrazide (AF647) using the single GUV method with CLSM. LfcinB stochastically induced local rupture in *E. coli*-lipid GUVs, causing rapid leakage of AF647; however higher LfcinB concentrations were required to induce a significant rate of local rupture in *E. coli*-lipid GUVs in comparison with *E. coli* cells and spheroplasts. To identify this reason, I examined the effect of membrane potential on LfcinB-induced local rupture of GUVs. To apply membrane potential to GUVs, I prepared *E. coli*-lipid GUVs whose membrane contains a monovalent cation channel, gramicidin A, and created K^+ concentration difference between the inside and the outside of the GUVs. I found that the rate constant of LfcinB-induced local rupture in GUVs increased greatly with increasing negative membrane potential. These results indicate that membrane potential plays a vital role in the LfcinB-induced local rupture of lipid bilayers and rapid permeabilization in *E. coli* plasma membrane. Based on these obtained results, I discussed the mode of action of LfcinB's antimicrobial activity and the effect of membrane potential.

(Chapter 3) In this chapter, I examined the effect of membrane potential on the action of LfB4-9. To reveal the location of LfB4-9 in cells and GUVs, I used a fluorescent probe lissamine rhodamine B (Rh)-labeled LfB4-9 (Rh-LfB4-9). First, I investigated the interaction of Rh-LfB4-9 with single *E. coli*-lipid GUVs containing AF647 and small GUVs comprising dioleoylphosphatidylglycerol (DOPG) and dioleoylphosphatidylcholine (DOPC) using the single GUV method with CLSM. I found that Rh-LfB4-9 entered the GUV lumen and bound to the membrane of the small GUVs without leakage of AF647, i.e., no pore formation or local rupture in the mother GUV. Using the same method of application of membrane potential to GUVs described in Chap. 2, I investigated the effect of membrane potential on the entry of Rh-LfB4-9 into single *E. coli*-lipid GUVs. I found that membrane potential greatly increased the rate of translocation of Rh-LfB4-9 into the GUV lumen and the rate of its entry increased with an increase in negative membrane potential. Next, I examined the interaction of Rh-LfB4-9 with single live *E. coli* cells and spheroplasts containing calcein using CLSM. I observed that in low peptide concentrations Rh-LfB4-9 entered the single *E. coli* cells and spheroplasts without leakage of calcein. A protonophore CCCP suppressed the entry of Rh-LfB4-9 into *E. coli* cells and spheroplasts. Based on these results, I discussed the effect of membrane potential on the action of Rh-LfB4-9.