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The Role of Tension on Antimicrobial peptide Magainin 2-Induced Pore Formation in Lipid Membranes

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学 位 論 文 要 旨

Abstract of Doctoral Thesis

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論文題目：

Title of Thesis：

The Role of Tension on Antimicrobial Peptide Magainin 2-Induced Pore Formation in Lipid Membranes

論文要旨：

Abstract：

Antimicrobial peptide (AMP) magainin 2 forms pores in lipid membrane region of plasma membranes and induces membrane permeation of the cellular contents, which is likely the main cause of its bactericidal activity. The mechanism of magainin 2-induced pore formation in lipid membranes is not clear, and controversial. On the other hand, it is known that lateral tension due to an external force induces pore formation in lipid membranes. There is a hypothesis that tension plays an important role in the magainin 2-induced pore formation; but there have been no experimental evidence. Here, we investigated the role of tension on magainin 2-induced pore formation in lipid membranes of GUVs.

<Chapter 2> To investigate the role of tension on magainin 2-induced pore formation in lipid membranes, we made experiments for the interaction of magainin 2 with GUVs both in the presence of external tension and also in the absence of tension. The binding of magainin 2 to a GUVs membrane increased the fractional change in the area of the membrane, δ, which was proportional to the surface concentration of magainin 2. This indicates that the rate constant of the magainin 2-induced pore formation (i.e., magainin 2-induced two-state transition from the intact state to the pore state), k_p, greatly increased with an increase in δ. The tension of a lipid membrane following aspiration of a GUV elevated magainin 2-induced pore formation. To reveal the location of magainin 2, the interaction of carboxyfluorescein (CF)-labeled magainin 2 (CF-magainin 2) with single GUVs containing a water-soluble fluorescent probe, AF647, was investigated using confocal microscopy. In the absence of tension due to aspiration, after the interaction of magainin 2 the fluorescence intensity of the GUV rim due to CF-magainin 2 increased rapidly to a steady value, which remained constant for a long time, and at 4-32 s before the start of leakage of AF647 the rim intensity began to increase rapidly to another steady value. In contrast, in the presence of tension, no increase in rim intensity just before the start of leakage was observed. These results indicate that magainin 2 cannot translocate from the outer

to the inner monolayer until just before pore formation in the membranes. Based on these results, we conclude that a magainin 2-induced pore is a stretch-activated pore and the stretch of the inner monolayer is a main driving force of the pore formation.

<Chapter 3> To understand the role of tension on charged GUVs, it is important to investigate the effects of electrostatic interactions in lipid membranes in the absence of peptides, because electrostatic interactions due to the negatively charged lipids play various important physiological roles. We, therefore, investigated the effects of the electrostatic interactions on the rate constant for tension-induced pore formation in lipid membranes of GUVs under constant applied tension systematically. A decrease in salt concentration in solution as well as an increase in surface charge density of the membranes increased the rate constant. These data indicate that the rate constant increases as the extent of electrostatic interaction increases. Based on the Poission-Boltzmann theory, we developed a theory on the effect of the electrostatic interaction on the free energy profile of the membrane containing a local rarefaction in the lateral density of the bilayer, i.e., a prepore. Based on the theory, the values of rate constant in various external tension has been obtained using the mean first passage time (MFPT) technique. The experimental results of the rate constant were well fit by the theoretical curves determined by MFPT in the presence of weak electrostatic interactions. Based on these results, we hypothesized that a decrease in the free energy barrier of the prepore state due to electrostatics is the main factor causing an increase in the rate constant.

<Chapter 4> We developed a theory on magainin 2-induced pore formation in lipid membranes. Our experimental results in chapter 2 show that the binding of magainin 2 in the outer monolayer increases the total area of the both monolayer of GUVs, and magainin 2 translocate from outer monolayer to inner one only after supercritical pore appearance in the membranes. These results suggested that the binding of magainin 2 in the outer monolayer induces tension in the inner monolayer of the GUV membranes. Using the theory developed in chapter 3, we obtained the k_p values of magainin 2-induced pore formation, which agreed with the experimental k_p values obtained in chapter 2.

Therefore, these investigations provide novel aspects of the role of tension on magainin 2-induced pore formation in lipid membranes.