Effects of Surface Charge Density of Lipid Membranes on the Pore Formation Induced by Antimicrobial Peptide Magainin 2: the Single GUV Method Study

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Interactions of antimicrobial peptides with lipid membranes have been investigated using a suspension of many small liposomes, and their details remain unclear. Recently we have proposed a novel method, the single GUV method; we observe and measure physical properties of single GUVs, and analyze these results over many single GUVs statistically, which will provide much new information that cannot be obtained by the conventional LUV suspension method [e.g.,1,2]. Using the single GUV method, we have succeeded in revealing elementary processes of the pore formation in lipid membranes induced by magainin 2 [3]. In this report, to elucidate the mechanism of the magainin 2-induced pore formation, we investigated the effect of surface charge density of membranes on the pore formation.

To change the surface charge density, we used GUVs of mixture membranes of negatively charged DOPG and electrically neutral DOPC, and controlled the DOPG concentration (mol%) in the membrane. The experiments were done in 10 mM PIPES (pH 7.0), 150 mM NaCl (buffer A) at 26 °C. We investigated the interaction of magainin 2 with single 60%DOPG/ 40% DOPC-GUVs containing the fluorescent dye, calcein, by fluorescence microscopy using the single GUV method. Low concentrations (0.5–5 μM) of magainin 2 caused the rapid leakage of calcein from single GUVs without change of the GUV structure, showing directly that magainin 2 forms pores in the membrane (Fig.1A,B) [3]. The rapid leakage of calcein from a GUV started stochastically, and once it began the complete leakage occurred rapidly (Fig.1C). The fraction of leaked GUV among the observed single GUVs, P_{LS}, increased with time. On the other hand, magainin 2 did not induce the leakage of calcein from single 30%DOPG/70%DOPC-GUVs at less than 10 μM. However, higher concentrations (≥10 μM) of magainin 2 induced a similar rapid leakage of calcein, indicating that the pores were formed in the membrane. The rapid leakage of calcein from a GUV started stochastically.

Figure 2A shows the dependence of P_{LS} after the interaction of magainin 2 with a single GUV for 6min on the magainin 2 concentration in the buffer for various GUVs with a different surface charge density. P_{LS} of DOPG/DOPC-GUVs with a same surface charge density increased with an increase in magainin 2 concentration. The magainin 2 concentration at P_{LS} = 0.5 increased with a decrease in the surface charge density. We can consider that the amount of magainin 2 bound with the membrane interface of GUVs decreased with a decrease in the surface charge density in the presence of the same magainin 2 concentration in the buffer, due to the decrease in the electrostatic attraction of magainin 2 with the membranes. Using the Gouy-Chapman theory,

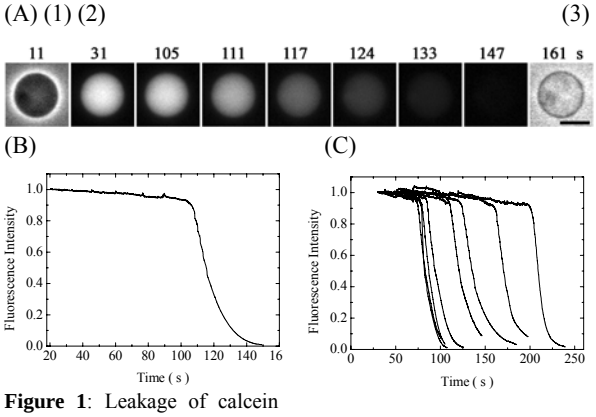

Figure 1: Leakage of calcein from single 60%DOPG/DOPC-GUVs induced by 3 μM magainin 2. (A) Fluorescence images (2) show that the calcein concentration inside the GUV progressively decreased after the addition of magainin 2. The numbers above each image show the time after the addition. Also shown are phase contrast images of the GUV at 0 (1) and at 161 s (3). The bar corresponds to 10 μm. (B) Time course of the change of the fluorescence intensity of the GUV shown in (A). (C) Other examples of change in the fluorescence intensity of single GUVs.

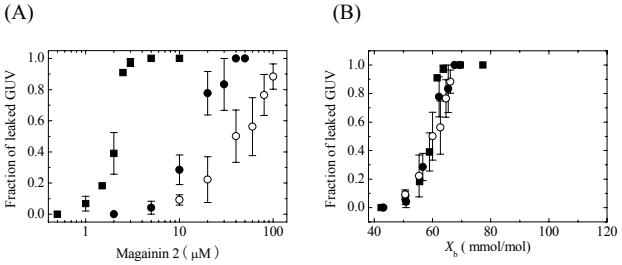

Figure 2: (A) Dependence of the fraction of leaked GUV, P_{LS}(6 min), on magainin 2 concentration in buffer A at 26 °C. (B) Dependence of P_{LS}(6 min) on magainin 2 concentration in the membrane, X_b, which were determined using values of K (110 for 30%DOPG/DOPC (○), 12 for 40%DOPG/DOPC (●), and 0.9 for 60%DOPG/DOPC-GUVs (■)).

we obtained the magainin 2 concentration in the membrane interface, i.e., the molar ratio of the magainin 2 bound with the membrane interface to lipids in the membrane, X_b. Assuming that the binding constant of magainin 2 with lipid membrane, K, depends on the DOPG concentration in the membrane, all the curves of P_{LS} vs. X_b were superimposed each other (Fig.2B). This result suggests that the pore formation of magainin 2 may be determined by the magainin 2 concentration in the membrane interface. In the Symposium, we will show the data of binding constant, and discuss in detail the relationship between the pore formation and X_b.

References

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