Form 1

2016 Report Form for Collaboration with Research Center for Biomedical Engineering

Year/month/date	
Number	2035

Date /Month/Year
date: 2017/03/31

To Chairman, Board of Directors, Research Center for Biomedical Engineering

Applicant
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Report Form for Collaboration Research

Research Theme	Interaction of antimicrobial peptide, magainin 2 with single bacterium.
Research Area	1. Biomaterials 2. Bioengineering ○3. Functional molecules 4. Chemistry/Electrical Engineering/Mechanical Engineering/Materials Science
Research Period	From: Date/month/Year To: Date/month/Year 1 / 6 / 2 0 1 6 ~ 3 1 / 3 / 2 0 1 7

Applicant Organization			
Name	Department	Title	Role
Dr. Md. Jahangir Alam	Dept. Biotechnology and Genetic Engineering, Islamic University	Lecturer	Experiments and analysis
Farliza Parvez	Grad. Sch. Sci. Tech., Shizuoka University	Ph. D. course student	Experiments
Md. Moniruzzaman	Grad. Sch. Sci. Tech., Shizuoka University	Ph. D. course student	Experiments
Masahito Yamazaki	Research Institute of Electronics, Shizuoka University	Professor	Analysis

Budget		
Travel Expense	Research Expense	Consumables
0 Yen	0 Yen	200,000 Yen

List of Equipments and Materials	Confocal laser scanning microscopy, Apparatus for the single GUV method acetoxymethylester calcein, carboxyfluorescein, Magainin 2 peptide
Collaboration Partners in the Research Center	Prof. Masahito Yamazaki (Shizuoka University)

Research Results (Including Purpose, Results, Figures, etc.)

1. Purpose of Research

Previously, to elucidate the mechanism of the bactericidal activity of magainin 2, we investigated the interactions of magainin 2 with lipid membranes using the single giant unilamellar vesicle (GUV) method, and revealed the elementary processes of magainin 2-induced pore formation in lipid membranes [1]. Recently we found that the magainin 2 induced pores is a stretch-activated pore [2]. In this report, as a consequence to the development of the mechanism of magainin 2 induced pore formations in lipid membrane, we investigated the interaction of magainin 2 with real bacterial membrane using single bacterium.

2. Results and Discussions

We investigated magainin 2-induced membrane permeation of calcein (Stokes-Einstein radius is 0.74 nm), from single *E. coli*. A typical experimental result of the effect of the interaction of 50 μM magainin 2 with single *E. coli* on the calcein concentration within the bacterium is shown in Fig. 1A. Prior to magainin 2 addition, the fluorescence intensity due to calcein inside *E. coli* was high (Fig. 1A (2) 0 s). During the addition of the magainin 2 solution, the fluorescence intensity inside the *E. coli* remained almost constant over the first 19 s, following which the fluorescence intensity decreased rapidly initially, then slowly decreased, and after 125 s, the fluorescence intensity became almost 0 (Fig. 1A (2), B), although a DIC image of the *E. coli* (Fig. 1A (3)) shows that the structure of *E. coli* remained intact. We can conclude that the decrease in fluorescence intensity results from the leakage of calcein from the *E. coli* through magainin 2-induced pores in the membrane. Thus, the time at which the fluorescence intensity began to rapidly decrease (t = 19 s) corresponds to the time of pore formation in the membrane. The same experiments were carried out using 20 single *E. coli* cells. The rapid leakage of calcein from an *E. coli* cell started stochastically, indicating that pores were formed stochastically. We also investigated the interaction of various concentrations of magainin 2 with single *E. coli* cells. We observed that magainin 2 induced leakage of calcein from the inside of single *E. coli*, and the time course of leakage greatly depended on magainin 2 concentration.

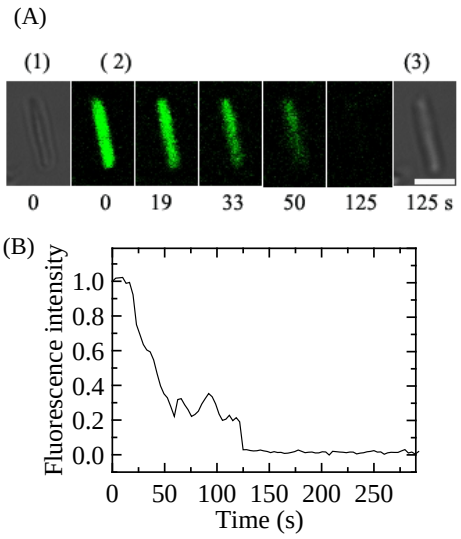

Fig.1. Interaction of 50 μM magainin 2 with single calcein-loaded *E coli*.

To clarify the target site of magainin 2 in *E. coli*, we measured the influx of the membrane-impermeant fluorescent probe, SYTOX green, into the bacterial cytoplasm in presence of magainin 2. We found that during the interaction of *E. coli* with magainin 2 its plasma membrane was damaged, and as a result, SYTOX green entered the *E. coli* through the damaged membrane. This suggests that magainin 2-induced bactericidality is due to damage of *E. coli* plasma membrane. Nevertheless, further investigations are necessary to know details about the interaction of magainin 2 with bacterial membrane.

3. References

(1) M. Z. Islam, J. M. Alam, Y. Tamba, M. A. S. Karal, M. Yamazaki, *Phys. Chem. Chem. Phys*. 16, 15752-15767 (2014).
(2) M. A. S. Karal, J. M. Alam, T. Takahashi, V. Levadny, M. Yamazaki, *Langmuir* 31, 3391-3401 (2015)

List of Publications Related to the Collaboration Research

1. J. M. Alam. M. Moniruzzaman, F. Parvez, and M. Yamazaki, Antimicrobial peptide magainin 2-induced leakage from single E. coli, Proceedings of the 18th of Takayanagi Kenjiro Memorial Symposium (Hamamatsu, 15-16, Nov., 2016), pp. 45-47.

List of Presentations (Conference, Meeting, etc)

1. J. M. Alam. M. Moniruzzaman, F. Parvez, and M. Yamazaki, Antimicrobial peptide magainin 2-induced leakage from single E. coli, Proceedings of the 18th of Takayanagi Kenjiro Memorial Symposium (Hamamatsu, 15-16, Nov., 2016)

List of Awards

The best presentation awards for young researcher:

1. J. M. Alam. M. Moniruzzaman, F. Parvez, and M. Yamazaki. Antimicrobial peptide magainin 2-induced leakage from single E. coli, Proceedings of the 18th of Takayanagi Kenjiro Memorial Symposium (Hamamatsu, 15-16, Nov., 2016).