Form 1

2019 Report Form for Collaboration with Research Center for Biomedical Engineering

Year/month/date	
Number	

Date /Month/Year
date: 6/March/2020

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

Applicant

Affiliation ; Jahangirnagar University
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Report Form for Collaboration Research

Research Theme	Effect of membrane potential on entry of cell-penetrating peptide Transportan10 into single vesicles.
Research Area	1. Biomaterials 2. Bioengineering **√3. Functional molecules** 4. Chemistry/Electrical Engineering/Mechanical Engineering/Materials Science
Research Period	From: Date/month/Year 14/05/2019 To: Date/month/Year 31/03/2020

Applicant Organization			
Name	Department	Title	Role
Md. Zahidul Islam	Department of Biotechnology and Genetic Engineering, Jahangirnagar University, Bangladesh	Associate Professor	Experiments and analysis
Md. Mizanur Rahman Moghal	Integrated Bioscience Section, Graduate School of Science and Technology, Shizuoka Univ.	Ph. D course student	Experiments
Farzana Hossain	Integrated Bioscience Section, Graduate School of Science and Technology, Shizuoka Univ.	Ph. D course student	Experiments
Masahito Yamazaki	Research Institute of Electronics, Shizuoka Univ.	Professor	Analysis
Collaboration Partners in the Research Center	Masahito Yamazaki		

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

1. Purpose of the research

In the report last year, we described the effect of membrane potential, $\Delta\varphi$, on entry of a cell-penetrating peptide (CPP), transportan 10 (TP10) into the lumen of single giant unilamellar vesicles (GUVs). The rate of entry of TP10 into single GUVs increased with negative membrane potential, $|\Delta\varphi|$, without pore formation in the GUV membrane [1]. To elucidate the effect of $\Delta\varphi$ on the interactions of peptides with GUVs in more detail, here we examined the effect of $\Delta\varphi$ on antimicrobial peptide (AMP)-induced damage of plasma membrane of bacterial cells and lipid bilayers [2]. As AMP, we selected lactoferricin B (LfcinB), because it is reported that LfcinB-induced damage of the bacterial plasma membrane is a key factor in its antimicrobial activity [3].

2. Results and discussion

First, we investigated the interaction of LfcinB with live single *E. coli* cells loaded with fluorescent dye, calcein, in the cytoplasm using confocal laser scanning microscopy (CLSM). LfcinB induced rapid calcein leakage from single *E. coli* cells, indicating that LfcinB induced damage or pore formation in the plasma membrane. To examine direct interaction of LfcinB with the plasma membrane, we investigated its interaction with single spheroplasts (loaded with calcein) derived from *E. coli* cells. LfcinB induced rapid leakage of calcein from spheroplasts. The presence of a proton ionophore, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) suppressed the LfcinB-induced leakage of calcein from single *E. coli* and spheroplasts. It is considered that CCCP dissipates $\Delta\varphi$ of cells, and thus, this result indicates the importance of $\Delta\varphi$ in the LfcinB-induced leakage from single *E. coli* and spheroplasts.

Next, we examined the interactions of LfcinB with GUVs of *E. coli* polar extract lipids (*E. coli*-lipid) containing fluorescent dye, AF647, in their lumens using the single GUV method. LfcinB induced rapid leakage of AF647 stochastically with some damage in the GUV structure, indicating that LfcinB induced local rupture in the GUV membranes. We obtained the rate constant of local rupture from its quantitative analysis. However, higher LfcinB concentrations were required to induce a significant rate constant of local rupture compared with *E. coli* cells and spheroplasts. To identify this reason, we applied $\Delta\varphi$ to the GUVs using K^+ concentration gradient, and examined the effect of $\Delta\varphi$ on LfcinB-induced local rupture. We found that the rate constant of LfcinB-induced local rupture in GUVs increased greatly with $|\Delta\varphi|$.

The results in this study show that the $\Delta\varphi$ greatly affects the LfcinB-induced rapid leakage from *E. coli* cells, their spheroplasts, and *E. coli* lipid-GUVs, indicating that $\Delta\varphi$ plays an important role in the LfcinB-induced local rupture or pore formation in the lipid bilayers and *E. coli* plasma membrane. On the basis of these results, we discuss the mode of action of LfcinB's antimicrobial activity.

3. References

[1] M. M. R. Moghal, M. Z. Islam, F. Hossain, S. K. Saha, M. Yamazaki, Biophys. J. 118, 57-69 (2020)

[2] F. Hossain, M.M.R. Moghal, M.Z. Islam, M. Moniruzzaman, M. Yamazaki, J. Biol. Chem. 294, 10449-10462 (2019).

[3] M. Moniruzzaman, M.J. Alam, H. Dohra, M. Yamazaki, Biochemistry, 54, 5802-5814 (2015).

List of Publications Related to the Collaboration Research

(1) Md. Mizanur Rahman Moghal, Md. Zahidul Islam, Farzana Hossain, Samiron Kumar Saha, and Masahito Yamazaki, Role of Membrane Potential on Entry of Cell-Penetrating Peptide Transportan 10 into Single Vesicles, *Biophys. J.* 118, 57-69, 2020

(2) Farzana Hossain, Md. Mizanur Rahman Moghal, Md. Zahidul Islam, Md. Moniruzzaman, and Masahito Yamazaki, Membrane potential is vital for rapid permeabilization of plasma membranes and lipid bilayers by the antimicrobial peptide lactoferricin B, *J. Biol. Chem.*, 294, 10449-10462, 2019.

List of Presentations (Conference, Meeting, etc)

(1) Farzana Hossain, Md. Mizanur Rahman Moghal, Md. Zahidul Islam, Md. Moniruzzaman, Masahito Yamazaki, "Membrane potential is vital for rapid permeabilization of plasma membranes and lipid bilayers by the antimicrobial peptide lactoferricin B", American Biophysical Society 64th Annual Meeting, San Diego Convention Center, San Diego, USA, 15-19, Feb., 2020.
(2) Md. Mizanur Rahman Moghal, Md. Zahidul Islam, Samiron Kumar Saha, Masahito Yamazaki, "Effect of membrane potential on entry of cell-penetrating peptide transportan10 into single vesicles", American Biophysical Society 64th Annual Meeting, San Diego Convention Center, San Diego, USA, 15-19, Feb., 2020.
(3) Farzana Hossain, Md. Mizanur Rahman Moghal, Md. Zahidul Islam, Md. Moniruzzaman, Masahito Yamazaki, "Membrane potential is vital for rapid permeabilization of plasma membranes and lipid bilayers by the antimicrobial peptide lactoferricin B", The 4th International Symposium on Biomedical Engineering, Act City Hamamatsu Congress Center, Hamamatsu, 14-15 Nov., 2019.
(4) Farzana Hossain, Md. Mizanur Rahman Moghal, Md. Zahidul Islam, Md. Moniruzzaman, Masahito Yamazaki, "Membrane potential is vital for rapid permeabilization of plasma membranes and lipid bilayers by the antimicrobial peptide lactoferricin B", The 57th Annual Meeting of Biophysical Society of Japan, 24–26 Sept., 2019, Miyazaki Seagaia Convention center, Miyazaki.
(5) Md. Mizanur Rahman Moghal, Md. Zahidul Islam, Samiron Kumar Saha, Masahito Yamazaki, "Effect of membrane potential on the entry of cell-penetrating peptide transportan10 into the lumen of single vesicles and its mechanism", The 57th Annual Meeting of Biophysical Society of Japan, 24–26 Sept., 2019, Miyazaki Seagaia Convention center, Miyazaki.

List of Awards

Registration of research-theme continuation for next year Prior consent from the collaboration partner in the Research Center is necessary.	Yes

Research plan for the next year (from April 1, 2020 to March 31, 2021), if the collaboration research is continued.

We want to continue and expand this project. As the research theme, we want to use a new title "Effects of membrane potential on action of antimicrobial peptides and cell-penetrating peptides".

Under this project, we have found so far the effects of membrane potential on actions of an AMP, LfcinB and a CPP, CF-TP10 and revealed clearly the parts of their elementary processes using the single GUV method. These studies have been just started. If we use the advantage of the single GUV method, we will get more information on the elementary processes of actions of AMPs and CPPs on membrane potential and their mechanisms. In the project at new fiscal year, we will investigate the effect of membrane potential on the action of an AMP, magainin 2 (mainly pore formation in the membrane), and the action of a CPP, LfcinB (4-9) (mainly the entry into cells and vesicles) from various aspects of the interactions of these peptides with single GUVs. We will also examine the physical properties of GUVs under membrane potential using various methods. The results of the effect of membrane potential on the action of these AMPs and CPPs are very helpful to develop new peptides with a bactericidal activity and novel vectors for the delivery of drugs, chemicals, oligonucleotides, and proteins into cells for curing various diseases.