# Report on the short-term overseas study program for KU Engineering students Graduate School of Engineering, Kyoto University

| Name: | Risa Naka | Date: | 2022/10/21 |
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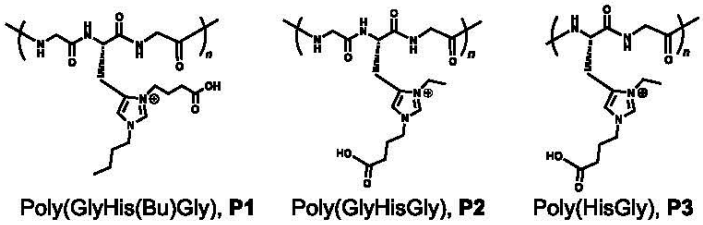
| Department/ Undergraduate School: | Material Chemistry | Grade: | M1 |

1. About my research

[Introduction]

In plant, cell wall function as a frame for them to maintain mechanical strength to survive in physiological conditions. Since cell wall has significant stretchability and physical strength, it is considered as an inexhaustible bio-resource to develop various material. However, cell wall is mainly comprised with cellulosic microfibrils that forms recalcitrant structure and hard to modify under controlled. Furthermore, this complicated-polar structure of cellulose network could be a physical barrier for nanocarriers to penetrate cell wall.

Thus, a novel method that enables to modify cell wall are now required from various field. Our laboratory newly developed zwitterionic polypeptide, 1st generation poly (GlyHisGly) (Fig.1 **P1**), that are zwitterionic modified (~55%) and can dissociate amorphous region of cellulose microfibrils. In addition, it enables cellulose to dissolve (dispersed) in water (zwitterionic polypeptide solution) and open the way to cell wall modification as material. However, this polypeptide still not have high cellulose interaction ability and requires higher concentration to loosen cellulose microfibril networks. Thus, to optimize the cell wall modification ability of polypeptides, we currently developed new two kinds of zwitterionic polypeptides, 2nd generation poly (GlyHisGly) (Fig.1 **P2**) and poly (GlyHis) (Fig.1 **P3**) that is highly zwitterionic modified (> 90%) among each monomer units. Therefore, in this study (during this stay) the cell wall interaction ability of 3 types of zwitterionic polypeptides (1st generation and 2nd generation) were quantitatively evaluated by measuring nano-scale and macro-scale mechanical property of zwitterionic polypeptide treated cell wall and to elucidate the key factor for polypeptides to interact with cell wall.




K. Tauchiya, *et al. Biomacromolecules*, 2020, *21*, 1785-1794.

Fig. 1 Chemical structure of zwitterionic polypeptides. P1: 1st generation poly(GlyHIs(Bu)Gly), P2: 2nd generation poly(GlyHisGly), P3; 2nd genetration poly(GlyHIs)

[Results and discussion]

1. Macroscale mechanical property changes of zwitterionic polypeptide treated cell wall

Onion epidermal cell wall was immersed under 2mg/ml zwitterionic polypeptide solution for 1h and clamped to conduct tensile test to be stretched with 3 mm/min. In addition, creep assay was also conducted to evaluate macro-scale mechanical property. From the results, treating with polypeptides slightly decreased the plasticity in cell wall. In addition, by treating with P1 and P2 increased the stiffness (and elongated the yielding region, Fig.2B, E). Considering these facts, it suggests the possibility that polypeptides allowed to increase interaction points (cross-

linking points) with cellulose microfibrils (or pectin) and limited the cellulose microfibrils to slip (plastic deformation).

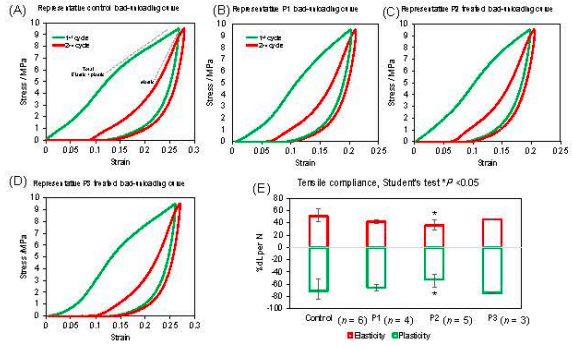


**Fig. 1.** Zwitterionic polypeptide effects on tensile mechanics of onion epidermal cell wall. (A-D) Representative load-unloading curve of buffer / zwitterionic polypeptide treated onion epidermal cell wall. For each dataset, the first extension is the upper curve which contain both elasticity and plasticity, while the second extension is the bottom curve containing only the elasticity of cell wall. These are representative curves with tensile compliance around the average. (A) buffer treated cell wall (B) P1 treated cell wall (C) P2 treated cell wall (D) P3 treated cell wall. (E) Statistical summary of elastic and plastic compliances (mean ±SE). Student's *t*-test (paired two-tail) was used to asses statistical significance (*$P$<0.05). The experiment was repeated two times with similar results.

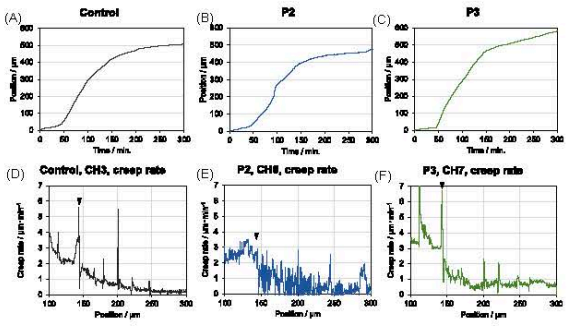


**Fig. 2.** Zwitterionic polypeptide effects on cell wall creep. (A-C) Representative creep curve of 2 mg/ml zwitterionic polypeptide treated onion epidermal cell wall. (A) 20mM HEPES buffer treated cell wall (B) P2 treated cell wall (C) P3 treated cell wall. (D-E) Representative creep rate curve of 2 mg/ml zwitterionic polypeptide treated onion epidermal cell wall. (D) 20 mM HEPES buffer treated cell wall (E) P2 treated cell wall (F) P3 treated cell wall

2. Nanoscale mechanical property changes of zwitterionic polypeptide treated cell wall

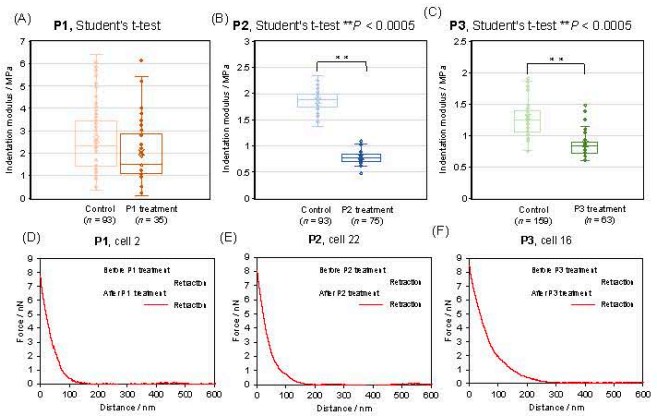


**Fig. 3.** Zwitterionic polypeptide effects on cell wall nanoindentation. (A-C) Statistical summary of indentation modulus. (A) P1 treated indentation. (B) (B) P2 treated cell wall (C) P3 treated rate curve of 2 mg/ml zwitterionic polypeptide treated onion epidermal cell wall. (D) 20 mM HEPES buffer treated cell wall (E) P2 treated cell wall (F) P3 treated cell wall

To evaluate the nanomechanical properties of zwitterionic polypeptide treated onion epidermal cell wall, onion epidermal cell wall was immersed under 2mg/ml zwitterionic polypeptide solution and indented. After treating with P2 and P3, cell wall surfaces were significantly softened and significantly decreased the indentation modulus. (Fig.3A, B) These results can be interpreted that the zwitterionic polypeptides multiply attached to the cross lamellar layer of cell wall (amorphous polysaccharide layer or between the cellulose microfibrils) and the cross lamellar surface were covered by soft matrix. Thus, the cantilever could indent deeper than the control to reach the same peak force.

[Summary]

Zwitterionic polypeptide P2 effected the cell wall nano/macro mechanical property the most meaning that P2 was the most effective peptide to interact with cell wall. Considering the decrease of indentation modulus in cell wall surface and decreasing the plasticity of tensile compliance and the creep rate of cell wall, the P2 might adhere between the cellulose microfibrils or to the embedded pectin layer. Thus, zwitterionic polypeptide might performed as a crosslinker so that the cellulose microfibrils disabled plastic deformation and also slightly decreased the creep rate in a quick response. For future plans, to elucidate which is the peptide interacting, amorphous cross lamellar

layer, or the cellulose microfibrils, the amount of pectin dissolved in zwitterionic polypeptide solution should be quantitatively measured to confirm the affinity between the pectin and zwitterionic polypeptides.

3. During my stay in Pennsylvania State University

Pennsylvania State University was located in the very calm city, university park, and Cosgrove lab where I commuted everyday was in the Frear north building (Fig. 2A). My lab member (Fig. 2B) kindly supported me a lot and welcomed me. During my stay, I had brunch with my friends on Saturday mornings and ate pancakes and omelette (traditional American breakfast, Fig.2C) The restaurant called "The Waffles shop" was crowded on weekends and we always needed to wait for an hour but still it was worth waiting. If you visited state college, I strongly recommend you going to this restaurant and enjoy American brunch.

Pennsylvania State University was famous for its American football team called "Nittany Lions". The football game was held on every Saturday and during my stay there were 3 big games in the beaver stadium, which is on campus (Fig. 2D). Before games, party called tailgate were always held and multiple people gathered outside of the stadium to kick the mood into gear. I also once joined the celebration party and enjoyed the mood. During the tailgate, music was played by the brass-band and what was surprising to me is that only men was playing in the band just like "ouendan" in Japan. I became interested in such cultural common point and hope to asking about it next time.

[Summary for my short-time training in the U.S.]

Overall, thanks to the support of everybody, I had experienced a lot of things that are unforgettable in my life. It was too short period for me to conduct all of the experiments that I was planning to do but still I made connections who could support my research during this study and they hope me to come back again. I now feel a little confidence to become a researcher who can compete in global because I have enhanced my English conversation skills during this study. However, at the same time, there were several points that I should overcome. For example, students in the U.S. never hesitated to ask questions during the seminar and were actively learning from the professor. In addition, they tend to cooperate with their cohorts a lot. Thus, to efficiently learn a lot from various fields I also would like to ask questions to professor and my colleagues. Since I am now motivated to cultivate my skill to become a cosmopolitan researcher in the near future, I would like to dispel my hesitation and try to communicate (discuss) with experts as much as I can. I also would like to keep in touch with my friends in Pennsylvania and would like to have (maybe) collaborative research in the future to create (or develop) something sensational.

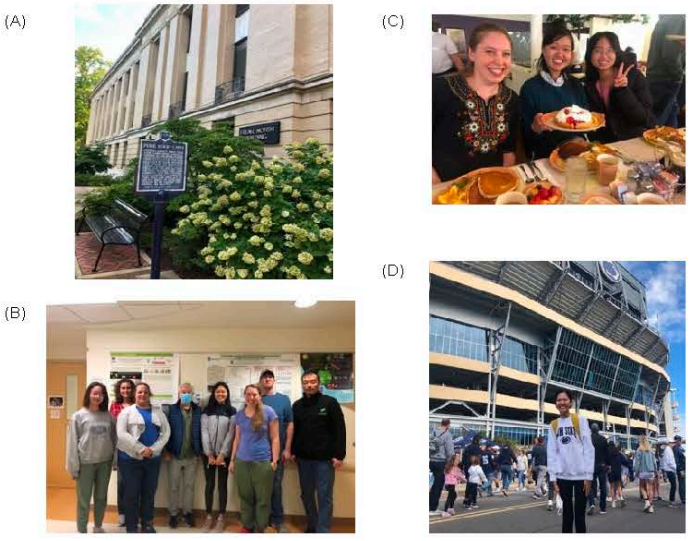



**Fig.4.** During my stay in Penn State (A) The building of my lab located. (B) Lab members. (C) Having brunch with my friends (D) Joining tale gate in front of beaver stadium