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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：
Title of Dissertation　　Molecular Biology and Materials Engineering of Poly-γ-glutamate
（ポリ-γ-グルタミン酸の分子生物学と材料工学）

学位論文要約：
Dissertation Summary

This doctoral thesis consists of five chapters. They are roughly classified into the two categories. The Chapter 1 and -2 show the molecular biology of poly-γ-glutamate (PGA). The Chapter 3, -4 and -5 deal with PGA engineering. The summaries and conclusion remark are presented as follows.

Chapter 1: Extra-chromosomal DNA maintenance in *Bacillus subtilis*, dependence on flagellation factor FliF and moonlighting mediator EdmS

PGA has been detected in various organisms, though the operon responsible for PGA synthesis has only been identified in bacteria. *Bacillus subtilis* possesses a choromosomally encoded PGA synthetic system that contains a cryptic protein of 55 amino acids, designed PgsE. We previously reported that PgsE affects the Zn^{2+}-dependent enhancer-like activity observed during PGA synthesis. Recently, we identified a candidate Extra-chromosomal DNA maintenance (EDM) mediator, resulting from the vector DNA-mediated transformation (or functionalization) of *B. subtilis* mutants. Unexpectedly, this inducer of EDM was found to be PgsE (from here on referred to as EdmS). It remains to be determined whether EDM in *B. subtilis* is dependent on this factor alone. In the Chapter 1, we investigate EDM in more detail in *B. subtilis* and analyze the roles of the flagellation factor - FliF and the moonlighting mediator - EdmS.

In *B.subtilis*, global regulators, such as ComA (predominantly for competence development), DegSU-DegQ (predominantly for proteolysis), SwrA (predominantly for swarming and *sigD* expression), and σ^D (predominantly for flagellation and chemotaxis), are responsible for PGA production. To investigate the potential effect of these global regulators on the process of EDM, *B.subtilis* regulator mutants were transformed with the pEFMS1 (a pWH1520 derivative carrying *B. subtilis edmS* gene) vector and R_m sensitive transformants were selected. Surprisingly, the EDM effect was absent in a *swrA* mutant(designated W619) derived from *B. subtilis* natural isolate ATCC6051. Interestingly, the influence of the *sigD* mutation on EDM induction in the natural isolate of *B. subtilis* differed from that in the laboratory strain. These findings suggested that in the laboratory strain σ^D activates the weaker, auxiliary promoter of the *fla/che* (flagellation and chemotaxis) operon and eventually operates as an anaplerotic effector for SwrA. Next, a *B. subtilis fliF* mutant (W954) was subjected to EDM analysis using a pEDMS1 vector. The results indicated that the EDM effect was lost in the absence of the FliF factor. To investigate this further, we constructed a pEDMS2 vector in which the EdmS and FliF factors were coproduced in the presence of xylose. Interestingly, the functions of the *fliF* mutant W954 and the *fla/che*-null mutant W834 were restored following the introduction of the pEDMS2 vector, suggesting that FliF is a crucial EDM cooperator.

The EDM factors - FliF and EdmS - do not possess any of the consensus (or characteristic) motifs associated with DNA-binding proteins or resemble any factors involved in the maintenance, replication, segregation, or copy-number

control of plasmids. Furthermore, these genes are chromosomally located in *B. subtilis* and are not carried on any natural plasmids identified to date. These findings therefore indicate the identification of a novel biological system for EDM.

A recent study reported that *Bacillus megaterium capE* (or *edmS*) gene (DDBJ accession no., AB544060) complemented the EDM phenotype in *B. subtilis*, while the *edmS* gene from *B. subtilis* complemented the EDM phenotype in *B. megaterium* cells. This suggested that *edmS* genes of the EDM system are transmissible between microbes, and can function in different host backgrounds, providing an interesting insight into the molecular mechanisms of EDM. These indicate a fundamental means for adaptation to the environment (or evolution) by some microbes by the uptake and in corporation of foreign EDM genes, and this may be demonstrated by the fact that the FliF orthologues are ubiquitous among prokaryotes.

Chapter 2: Cooperative adsorption of critical metal ions using archaeal poly-γ-glutamate

Chapter 2 reveals the adsorption capacities of L-PGA and two water-soluble synthetic polymers, i.e. polyacrylate (PAC) and polyvinyl alcohol (PVA), towards six industrially valuable metal ions (Co^{2+}, Ni^{2+}, Mn^{2+},Ga^{3+}, In^{3+}, and Dy^{3+}). The adsorption profiles of these polymers were also analyzed using the non-ideal competitive adsorption (NICA) model.

Several studies have been published pertaining to the chemical adsorption of divalent cations by water-insoluble (solid) materials bearing carboxylate moieties. However, the water-soluble polymers tested in the current study showed apparently little or no adsorption of Co^{2+}, Ni^{2+} and Mn^{2+}, indicating difficulty in the spontaneous transfer of the divalent metal ion-carboxylate polymer complexes into the solid phase. This difficulty could be attributed to negligible conformational changes to their separable (water-insoluble) complexes. In contrast, L-PGA and PAC showed good potential for the adsorption of Ga^{3+}, In^{3+} and Dy^{3+}, suggesting that their complexes to trivalent cations could be readily separated from aqueous solutions without using ultra- and nanofiltration procedures. These results therefore imply that anionic polyelectrolytes exhibit beneficial valence-dependent selectivity (viz., water-soluble polymers bearing multiple carboxylates) for metal ions.

Chapter 3: Poly-γ-glutamate-based Materials for Multiple Infection Prophylaxis Possessing Versatile Coating Performance

PGA possesses a nylon-like backbone bearing numerous polyacrylate-like carboxylate groups, making it highly soluble in water. In this Chapter 3, the effective synthesis and structural analysis of some water-insoluble PGA ion-complexes (PGAICs) using cationic surfactants, hexadecylpyridinium (HDP), dodecylpyridinium(DDP), benzalkonium(BZA) and benzetonium(BZT), were examined. Because PGA has potential for use as surface-contact adhesives, we were interested in discovering whether or not PGAIC retains the original function of PGA. Some material surfaces-coating tests were carried out using PGAICs dissolved in ethanol (0.1 wt % each), and the solvent that contains only 0.1 wt % partner surfactant was used as control. In the first experiment, the PGA/HDP-, PGA/DDP-, PGA/BZA-, PGA/BZT-, and partner surfactants-coated polypropylene (PP) disks were placed on every bed of a pathogenic bacterium (*Staphylococcus aureus*) and a prevalent species of *Candida albicans* in an agar plate. The assessment of antimicrobial performance was based on the presence of the zone of growth inhibition (also known as halos) around the disks. Next, the PGAIC- and partner surfactant-coated stainless steel and bathroom tile. sheets were used for the tests, and their anti-staphylococcal and anti-fungal activities were assayed as well. The results from the tests against *S. aureus* revealed that, regardless of a variety of materials, PGAICs retained surface antimicrobial activity, even after the water-soaking treatment.

Chapter 4: Effective elimination of water-borne *Escherichia coli* using archaeal poly-γ-glutamate-based

materials

Chapter 4 describes the synthesis of a novel bacteria-elimination material, i.e. PGAIC-coated active carbons (PGAIC-AC). In this Chapter, the potential of the PGAIC-AC system was also examined using *Escherichia coli* JM109 as a laboratory model of a water-borne pathogenic bacterium because *E. coli* is used worldwide as a fecal indicator species to assess the quality of (drinking) water.

An *E. coli*-elimination test was performed using the PGAIC-AC–embedded column. The water-borne *E. coli* cells appeared to be eliminated by passing through the column. PGAIC-ACs with different amounts of PGAIC coating were then applied to the column system, and the elimination tests were repeated. An increase in the amount of PGAIC coatings resulted in an increase in the *E. coli* elimination ratios, implying that, unlike the use of PGAIC-AC as a dispersant, bacterial elimination could be controlled in a dose-dependent manner when PGAIC was used as a functional carrier on the closed spaces in such column-like structures. Viable *E. coli* cells were virtually eliminated (> 99.9%) from the laboratory model of highly contaminated water (~ 2.0×10^4 CFU/mL) using our PGAIC-AC–embedded column.

Chapter 5: Engineering antimicrobial coating of archaeal poly-γ-glutamate-based materials using non-covalent crosslinkages

Chapter 5 shows a new strategy to introduce multiple non-covalent crosslinkages into the essential architecture of PGAIC to develop engineered materials with excellent chemical durability. A sustainable PGAIC material was actually synthesized using a *bis*-QA (quinolinium) compound used in medicinal lozenges, called dequalinium cations (DEQ^{2+}). In this Chapter 5, the potential of DEQ-bound PGAIC (PGA/DEQ) materials as durable antimicrobial coatings is compared with that of traditional HDP-bound PGAIC (PGA/HDP) materials.

In the present study, several crosslinker candidates were used to synthesize PGAICs. Diamine-type compounds (e.g., hexamethylene (viz., aliphatic) and phenylene (viz., aromatic) did not meet the requirements for PGAIC materials, whereas *N, N'*-hexamethylenebis (4-carbamoyl-1-decylpyridinium) (BDP^{2+}, a *bis*-pyridinium compound) was applicable, as was HDP^+ (a *mono*-pyridinium compound). DEQ^{2+} (a bis-quinolinium compound) was useful as well, but mono-quinolinium compounds were not preferred for the formation of water-resistant complexes. This implies unexpected performance (e.g., extreme solvent resistance and sustainable functionality) in PGA/DEQ as a novel PGAIC product with multiple, non-covalent crosslinkages. The “soakage” tests for PGA/DEQ actually indicated a dramatic improvement in the resistance of PGAIC materials to all the organic solvents tested.

PGA/HDP coatings on the plastic surfaces were stable during water-soaking; however, they were readily removed by the indicated organic solvents and concentrated salt solutions. In contrast, the PGA/ DEQ coatings on the same materials had excellent durability against all the solvents (or chemicals) tested. The morphology of PGAIC-coated microfibers on these plastic surfaces was further observed using a scanning electron microscope (SEM). The transformation of PGAIC into durable structures led to the further sustainable functionality of the antimicrobial coatings. The antimicrobial performance of the PGA/HDP coatings disappeared after soaking in the indicated solvents other than water, whereas a PGA/DEQ-coated disk resulted in the dramatic suppression of microbial proliferation (white bars) under any treatment conditions. Compared with the normal proliferation of Escherichia coli (~1.3×10^9 colony-forming units (CFU)), the log-reduction scores of PGA/HDP and PGA/DEQ coatings (after treatment) were estimated to be almost zero and approximately nine, respectively. These PGAIC coatings could, therefore, contribute to the microstructure-based polymer engineering of antimicrobial (bioactive) coatings.

Conclusion: The Chapter 1 and -2 revealed the common function of EDM among *Bacillus* PGA producers and proved the cooperative adsorption of archaeal PGA for critical metal ions. As described in the Chapter 3, -4 and -5, I studied on PGA application. In fact, PGA-based thermoplastic nanofibers,

antimicrobial coatings, and metal-binding gels were developed. The potential of PGA as advanced biomaterials, therefore, will be beneficial in sustainable materials engineering.

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