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Development of a method to dramatically improve the degradation rate

of the poly(ethylene terephthalate)-degrading enzyme PETase

-Outcomes leading to the practical application of biorecycling used PET products-

A research group including Professor Kenji Miyamoto and Assistant Professor Norifumi Kawakami and Mr. Makoto Furukawa of Keio University and Professor Emeritus Kohei Oda of Kyoto Institute of Technology carried out research aimed for improving the activity of a poly(ethylene terephthalate)-degrading enzyme (PETase), which they were the first to discover. The group successfully improved the degradation rate by more than a 100-fold by simple addition of a small amount of surfactant.

Poly(ethylene terephthalate) (PET) is used around the world as a material for plastic bottles, clothing, etc. Some PET products are being recycled, but there have been calls for a degradation technology with minimal environmental impact. The outcomes of this research can dramatically improve the PET degradation rate of the enzyme through a simple operation, which is anticipated to help realize the practical application of biorecycling technology for used PET products.

The findings of this research are published in the European scientific journal "ChemSusChem" and selected as the cover of the issue.

1. Main Points of Research

- Discovery of a method to dramatically improve the activity of a PET-degrading enzyme (PETase)
- · Solution of the mechanism that leads to improved activity

2. Background of Research

In recent years, the demand for plastic resins has been rapidly increasing and they have become indispensable materials for everyday life. However, this has led to environmental problems because they accumulate throughout the natural world without degrading, and there is a pressing need to resolve this issue.

To date, this research group has discovered a bacteria called "*Ideonella sakaiensis*" that relies on poly(ethylene terephthalate) (PET) as a source of nutrition and has identified an enzyme, PETase, which shows high specificity to PET (Reference 1). PETase is attracting attention as an enzyme that could solve environmental pollution caused by the disposal of PET, but its activity rate is low and not yet effective for practical application.

For the enzyme to degrade PET, first, it must come in contact with PET to start a reaction. One reason why the activity rate of PETase is low at this point can be attributed to the respective properties of PETase and PET. While PETase is hydrophilic, PET surface is hydrophobic, thereby

making contact between the two difficult. This research therefore aimed to increase the activity rate of the enzyme by improving the contact frequency between PETase and PET.

3. Content of Research and Results

The research group thought that if the PET surface could be coated with amphiphilic molecules, surfactants, the contact rate of PETase could be improved (Figure 1). They explored the use of surfactants as molecules to mediate the interaction between the hydrophilic PETase and the hydrophobic PET. Typical surfactants such as those used in detergents were selected as additives and under conditions including the surfactants, their influence on PET degradation activity was investigated. It was found that the addition of charged surfactants negatively considerably improved the activity rate, with the greatest achieved being over a 100-fold increase. Through this method, for which the required concentration of the surfactant was extremely low at 0.005%, it is possible to accelerate PET degradation very easily and efficiently.

Furthermore, despite the surfactant is a denaturant of the enzyme, PETase activity was still remained even after 36 hours of reaction. After 12 hours of reaction, the PET surface became clouded as shown in Figure 2, and when examined using a microscope, countless scratches were observed. Additionally, after 36 hours of reaction, the PET thickness was decreased 20%. The rate of activity during this timeframe, despite the reaction conducted at 30°C, was equivalent to those of thermostable enzymes that require high temperatures of 70°C or greater, making this the first demonstration of the possibility of efficient PET degradation at ambient temperature.

a) PETase Hydrophilic PET Hydrophobic b) Surfactant MMMM PET PET surface hydrophilization Accelerated the interaction

Figure 1. a) A problem in PET degradation. b) The concept of this research.



Figure 2. Hydrolyzed PET surface after 0 h or 12 h of reaction.

4. Future Developments

When compared with chemical degradation methods for PET, degradation through enzymes has a lower energy consumption and is an environmentally friendly process. It is thought that the high rate of activity achieved through the findings of this research could open up possibilities for the practical application of PET biorecycling. <Reference 1>

Title: A bacterium that degrades and assimilates poly(ethylene terephthalate)

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< Details of Original Paper>

Title: Acceleration of Enzymatic Degradation of Poly(ethylene terephthalate) by Surface Coating with Anionic Surfactants

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<Glossary >

%1 Enzyme: Proteins produced by organisms to metabolize substances.

- *2 Hydrolysis: When an ester bond is degraded with a water molecule, alcohol and a carboxylic acid are produced.
- 3 Surfactant: A generic term for substances that have a hydrophilic part and a hydrophobic part within each molecule and is widely used as a detergent, etc.

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