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Author(s)	Pilakka Veedu, Anju
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# SUMMARY OF DISSERTATION

## Functional modification of mussel adhesive proteins for environmental applications

By

ANJU PILAKKA VEEDU



Laboratory of Biotechnology for Resources Engineering,

Division of Sustainable Resources Engineering,

Graduate School of Engineering,

Hokkaido University, Japan

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# Functionalization of mussel adhesive proteins for environmental applications

Mussel adhesive proteins (MAPs) are produced by marine organism, mussel, and it shows strong adhesive property underwater. Many adhesive proteins are found in the mussels' foot, a tough elastic fiber that helps them to attach to surfaces in the marine environment. These special adhesive proteins can withstand heavy water flow, temperature changes, and saltiness that allow mussels to anchor firmly to various surfaces rocks, wood, and metals. Moreover, it is found that these bio-adhesives can adhere to inorganic and organic surfaces like titania, mica, silica, and plastic. Another important feature of MAPs is their biocompatibility and biodegradability. Because MAPs have been shown to be non-toxic and non-immunogenic, they have been investigated as potential material for tissue engineering, drug delivery, and wound healing. MAPs have also been studied for their potential environmental applications due to their strong affinity for heavy metals, dyes, and other pollutants, which makes them useful to remove contaminants from water and soil. The production of MAPs by recombinant protein production is common because this way the natural properties of the protein are retained, and it is an environmentally friendly method. However, there are some limitations to recombinant production of MAPs, such as incorrect folding, formation of inclusion bodies, or protein aggregation, whereby the protein loss its natural structure. Moreover, MAPs' quick adhesive properties make the process more difficult by losing protein during production due to their quick adhesive properties. If this problem is solved, we can achieve a higher yield of a strong underwater adhesive. Additionally, achieving control over the adhesive properties can contribute to switching on adhesion to various surfaces.

The dissertation presents functional modifications of mussel adhesive proteins and their applications in environmental fields. This research work aimed to overcome the limitations of the production of MAPs and improve adhesive properties by genetic modification. The proposed production method in this research can be used for scaling up the production of MAPs and easy handling in addition to improved adhesive behavior. It was found that the functionalized MAPs with higher solubility in water and control over adhesive properties can be used for controlled agglomeration of inorganic and organic materials and their significance in environmental applications was tested. In addition to the functionalized MAPs with high solubility, novel functionalized MAPs that can switch adhesion behavior upon responding to stimuli also developed.

Chapter 1 presents the research background, introduction to MAPs, their adhesion mechanism, research scope and the objective, originality, and significance of the research were discussed.

In Chapter 2, previous literatures about recombinant MAPs are reviewed, and their functional modifications for application or property demands was discussed.

In Chapter 3, the proposed research aims to address the limitations in the production of recombinant MAPs with a soluble protein tag. By fusing a soluble protein tag to MAPs, we expected solubility improvements and reduced aggregation of the protein, which are great challenges in the production of MAPs, as well as controllable adhesive properties. The study focuses on Fp1, one of the representative MAPs found in mussel cuticles, which is composed of 12 repeating units of a decapeptide ((AKPSYPPTYK)<sub>12</sub>). We achieved the solubilization of MAPs with controllable adhesive properties by functionalizing them with a soluble protein, InaKC. To evaluate the controllable adhesive property of the fusion protein InaKC-Fp1, we used magnetite particles as a model material and found that Fp1 exhibited restored adhesive properties after removal of InaKC tag, leading to the agglomeration of magnetite particles underwater. The findings of our study hold significant importance for the production of functional MAPs on a larger scale with controlled adhesion properties. Since many biological and environmental processes occur underwater, there is a high demand for sustainable and biocompatible bio-adhesives to replace petrochemical-based adhesives in industrial applications, particularly in the biomedical and environmental fields. Furthermore, our research opens avenues for future investigations into the controlled agglomeration of micro/nanoparticles underwater, which has crucial implications in the biomedical and environmental sectors.

Chapter 4A focused on the application of InaKC-Fp1 fusion protein for controlled agglomeration of organic and inorganic particles together. Since Fp1 can adhere to organic and inorganic surfaces underwater, we utilized this special property for the recovery of microplastics from an aqueous environment. In the preliminary studies, InaKC-Fp1 showed controlled agglomeration of polystyrene (PS) microparticles after removing the InaKC part from the fusion protein. Then, the agglomeration of PS (organic) and magnetite (inorganic and magnetic) particles using Fp1 was examined. The Agglomerate of PS particles with magnetite was formed underwater efficiently by the strong adhesive property of Fp1, and the agglomerate could be recovered by magnetic power. Our findings revealed an excellent recovery rate of

99.6% using the Fp1-mediated magnetic recovery method. The approach using non-toxic, biocompatible, and biodegradable material for the removal of microplastics has an important implication in the field of sustainable environmental applications. Additionally, it provides a simple and efficient separation process without the need for complex equipment or chemicals.

The magnetic recovery of microplastic using our technique requires the separation of magnetite from microplastics. This motivated us to develop stimuli-responsive MAPs in Chapter 4B, which can switch adhesive properties responding to oxidation and reduction stimuli. In this research, we developed stimuli-responsive MAPs by functionalizing them with cysteine amino acids. We expect that Fp1, which has a smaller repeating unit (6 repeating units of decapeptide), will be less adhesive than MAPs with 12 repeating units (which was previously used during the studies in chapter 3), and will be able to agglomerate microplastic and magnetite together under oxidative stimuli by forming strong disulfide bond and improving adhesive properties. After reductive stimuli, the microplastics will be separated from the magnetite due to the debonding of disulfide linkage. The reuse of Fp1 protein, recovered microplastics and magnetite will be possible in this way. In the experimental studies, the Fp1 with Cysteine tag showed stimuli-responsive adhesive nature. Moreover, adhesive studies were carried out on different types of plastic surfaces like PTFE, PE, PET, and PS. This cysteine-functionalized Fp1 will be a great addition to the stimuli-responsive MAPs list since it is a simple method, and since the produced was smaller repeating units of Fp1, higher productivity will be achieved compared to larger Fp1 with many repeating units of amino acid sequence.

Chapter 5 summarizes all the results achieved in this study. The future scope of the study will be utilizing the stimuli-responsive MAPs for different bioremediation applications. By further exploring the functionalization of MAPs it can be utilized in environmental remediation efforts. These proteins can be designed to selectively bind and remove pollutants from water sources, such as heavy metals or organic contaminants. They can also be used to create smart membranes for water filtration, improving water quality and addressing water scarcity issues. This can be achieved by designing novel MAPs which respond to desired environmental stimuli needed for specific applications. The biodegradable sustainable strong underwater adhesive MAPs are still not yet fully studied and utilized for potential applications it can perform well, and I hope this work can be a foundation for many future underwater adhesives based on MAPs for environmental applications.