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論 文 要 旨

THESIS SUMMARY

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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

In-cell protein crystals appear with various functions, and high stabilities have been drawing much attention to use as the scaffold for constructing solid functional materials. In this thesis, I broaden the use of in-cell protein crystals to build functional solid materials with novel approaches: releasing protein assembly from engineered crystals and immobilizing protein assemblies into protein crystals.

In Chapter 2, filament protein assemblies were built using an engineered TbCatB crystal produced by an insect cell, sf9. TbCatB crystal structure provides a potential platform for screening the ability to build diverse filament protein assemblies. Cysteine mutations were introduced into TbCatB to explore the creation of various filament protein assemblies by generating disulfide connections via the cross-linked reaction between two rationally chosen cysteines. In the mutant R91C/T223C, the purified crystals can spontaneously form the disulfide bonds without additional oxidation reaction, creating long double-fibril proteins. According to a crystallographic study, the disulfide bonds of this design did not develop when the crystal was still inside insect cells. After being extracted from the cell, the crystal can spontaneously form disulfide bonds via a cross-linked reaction in response to environmental stimuli. Unique double filaments were formed by dissolving the pure crystal at pH 3, which is higher than the design assumption for single filament protein assembly. The crystalline environment of TbCatB crystal offers a robust intercellular contact network between two nearby filaments formed by the disulfide bond network.

A unique approach for immobilizing protein assembly into polyhedral crystal (PhC) was established in Chapter 3. Various types of proteins have been immobilized using in-cell protein crystals as a platform. However, most studies focused on proteins that remained monomeric. Immobilization of protein cages into in-cell protein crystals remains difficult. Furthermore, protein assemblies, such as protein cages with an interior chamber and a surface, serve as a scaffold for functionalization, and the outside surface can be changed to allow protein crystals to be immobilized. As a result, if a protein cage can be adsorbed into a protein crystal while retaining the cage structure, the application to manufacture solid materials employing in-cell protein crystals can be expanded. This study devised a method for immobilizing a typical protein cage, ferritin (Fr), within a PhC crystal. To create a hybrid crystal PhC immobilized H1-Fr cage, the H1 segment produced from PhC was fused to the N-terminal of Fr (H1-Fr) and co-expressed with polyhedrin in the same E.coli cell. In E.coli

cells, H1-Fr can self-assemble to form a cage structure with 24 H1 segments on the exterior surface of the Fr cage. The many H1 segments on the outer surface give multiple points of interaction with polyhedrin, resulting in effective H1-Fr cage immobilization inside the PhC crystal. In contrast, only WTFr cage co-expression with PhC cannot be immobilized into PhC crystal. The hybrid crystal H1-Fr/PhC characterization shows that H1-Fr cages were immobilized near the surface of the hybrid crystal, retaining the crystallinity of PhC. This data is promising for further functionalization study of H1-Fr/PhC solid materials. Furthermore, the controllable releasing of the H1-Fr cage feature against various pH might help construct pH releasing drug delivery system.

Another method for encapsulating protein assembly employing a protein needle (PN) as an assembler was devised in Chapter 4. This approach was meant to be used for proteins that remained monomeric after production. The protein needle (PN) is a protein created by our group that has the ability to build a stable trimer structure. Using this characteristic, I fused the target protein, including the H1- segment, H1-sfGFP, to the N-terminus of PN and co-expressed it with polyhedrin in the same E.coli cell to produce the hybrid crystal PhC encased H1-sfGFP-PN. The PN trimerization feature resulted in the accumulation of three H1-sFGFP molecules surrounding the PN body, resulting in numerous point contacts of the H1 segment with polyhedrin, which is expected to increase sfGFP encapsulation yield when compared to previous reports of H1-sfGFP and polyhedrin co-expression.

In addition, parameters influencing the high encapsulation yield of the design H1-sfGFP-PN into PhC crystal were identified. Aside from the various H1 segment contacts, quantitative study shows that PN morphology (surface charge and shape) has a considerable impact on target protein encapsulation. Furthermore, a synergy between the His-tag cluster at the C-terminus of PN and numerous H1 segments on encapsulation yield increase has been found. The pH dependence of releasing H1-sfGFP-PN in the hybrid crystal H1-sfGFP-PN/PhC suggests that it could be useful for biological applications such as the development of solid vaccines.

In summary, I have established unique methods to construct protein assemblies and effectively immobilize protein assemblies into in-cell protein crystals for both natural protein cages or proteins that stay in monomeric tage. Furthermore, the methods are promising to expand to various proteins with similar properties and are expected to construct functional solid material using in-cell protein crystals.