論文 / 著書情報
Article / Book Information

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Title(English)	Engineering of protein crystals for development of artificial biomaterials
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学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工学 系 生命理工学 コース	申請学位（専攻分野）： Academic Degree Requested	博士 (工学) Doctor of
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

This thesis entitled "Engineering of protein crystals for development of artificial biomaterials" aims to construct the functional materials by utilizing protein crystals. The protein crystal structure promotes the molecular design for controlling the desired interactions for the development of artificial materials. The porous structures of protein crystals induce the accumulation of foreign proteins.

Chapter 1 describes the general introduction for the construction of artificial protein assemblies and the utilization of protein crystals as functional solid-materials. Recently, the development of protein assemblies has attracted much attention in the fields of biomaterial sciences. Protein crystals are promising materials for designing artificial protein assemblies due to their highly-ordered structures and their typical characteristics that are completely different from protein in aqueous solution. Notably, the intracellular protein crystals are considered as attractive solid-materials because the proteins can spontaneously be crystalized to form the stable crystals in living cells. In this study, engineering of protein crystals creates different functional materials, including the supramolecular assemblies and solid-containers for accumulating foreign proteins.

Chapter 2 describes the construction of supramolecular protein by engineering protein crystals. A protein termed as RuBisCO was employed to develop a new method to generate the tube assembly structure formed in the crystalline lattice environment although the crystals were dissolved. Specifically, the protein nanotubes of RubisCO were released after cross-linking protein crystals to stabilize the desired assembly structure via disulfide bonds, followed by the dissolution of the crystals. The formation of nanotubes was demonstrated to depend on the Cysteine (Cys) residues located the interface of monomer in the crystal, types of cross-linkers, and concentration of cross-linkers. The mutant with the introduction of Cys could generate the tube structure after cross-linking and dissolution while it was restrained to form the tube structure from the solution of protein under the same conditions. This study suggests that it is potential for the construction of different types of supramolecular assemblies by engineering protein crystals.

Chapter 3 describes the utilization of protein crystals termed as polyhedral crystalized in insect cells for the construction of a solid-biocatalyst for a cascade reaction by immobilizing two kinds of enzymes within insect cells. When the foreign enzymes fused with a tag peptide of H1 were co-expressed with polyhedral protein, the polyhedral crystals containing enzymes were synthesized in insect cells. Under this feature, a hollow cage structure was designed to enhance the dual-immobilization and promote the chemical reaction. The large holes within the mutant crystals could enhance the uptake of foreign enzymes in comparison with the wild-type protein crystal. As a result, the enzymatic activity for the cascade reaction was improved approximately 2-fold higher than that of the wild-type crystal. Therefore, it was demonstrated the potential for constructing the solid-catalyst for one-pot reaction by immobilizing enzymes within the polyhedral crystals in the intracellular environment. This study suggests that the engineering of protein crystal within cells supports foundations for the construction of solid-catalysts with high efficiency, high stability, and potential for recycling.

Chapter 4 describes the construction of multi-layered structures within intracellular protein crystals by immobilization of two fluorescent proteins. The immobilization of foreign proteins was induced by fusing H1 tag peptide into foreign proteins as similar in chapter 3. Specifically, the new system for expression and controlling the expression of proteins in *E. coli* was constructed. The co-expression of fluorescent proteins and polyhedral protein in E. coli induced the immobilization of foreign proteins into polyhedral crystals in the intracellular environment. The formation of layered structures was controlled by changing temperature, types of inducers, and expression time. It was found that the layered structures were observed with different ratios at the surface and inside of polyhedral crystal. This method opens a new strategy for positioning of target foreign protein into typical localizations that is considered as an important feature for the development of cascaded-catalysts and drug delivery system.

Chapter 5 summarizes the achievements from chapter 2 to chapter 4 and their contribution to the field of biomaterial science. The functional protein crystals have been constructed by the utilization of immobilizing foreign proteins into polyhedral protein crystals to generate the solid-biocatalysts and multi-layered materials in the intracellular crystallization environment. Furthermore, the design of the molecular interface of protein crystals is useful to create the pore-expanded protein crystals and new assemblies of proteins after dissolution. Applying the methods from this thesis to various protein crystals can open new routes for the development of not only solid-catalysts but also desired supramolecular assembly structures as artificial biomaterials.

In summary, this thesis reports the achievements and provides findings on the creation of functional biomaterials using protein crystals. Furthermore, the strategies and related results have great contributions to engineering and industry.