DEVELOPMENT OF FUNCTIONAL PROTEIN POLYMERS PREPARED BY A LACCASE-CATALYZED CROSS-LINKING REACTION

ダニ, ペルマナ

（様式２）

氏　　名　：　ダニ ペルマナ

論 文 名　：　DEVELOPMENT OF FUNCTIONAL PROTEIN POLYMERS PREPARED BY A LACCASE-CATALYZED CROSS-LINKING REACTION
（ラッカーゼが触媒する架橋反応を用いた機能性タンパク質ポリマーの開発）

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論 文 内 容 の 要 旨

Proteins nowadays are used in more wider fields, ranging from catalysis to nanoprobes in diagnostic applications. The limitation on the structure as well as the function of proteins could be improved by engineering them to create artificial enzymes or to form polymeric enzymes. Preparation of polymeric proteins as well as enzymes can be done by engineering the proteins using some strategies such as genetic modification via tandem fusion or site-specific cross-linking reaction of proteins. Polymerization of proteins to form protein polymers has been developed to expand the functionalities of proteins or to create a unique and novel property of the conjugates.

In Chapter 2, the TL-catalyzed cross-linking reaction of Y-tagged proteins was studied to investigate the possibility of TL as an enzyme for polymerization reaction of proteins. TL was able to catalyze cross-linking reaction of Y-tagged proteins specifically to yield protein polymers. Unlike small substrates, the TL-catalyzed protein cross-linking reaction occured efficiently at basic pH conditions but not in the acidic conditions. The BAP/pG_2pA polymers made by TL showed higher functionalities than those made by HRP. The results described here showed the potential of TL as an another enzyme for HRP to perform protein polymerization. Also, the need of controlling cross-linking degree of the protein polymers was revealed in this study and TL possesses capabilities of achieving functional protein polymers with different cross-linking degrees, which will open up the utilization of TL into biomedical and diagnosis fields of studies.

In Chapter 3, a new strategy for the polymerization of HRP was demonstrated by creating Y-tagged HRPs and conducting polymerization of HRPs through the Y-tags using laccase. Compared with the self-cross-linking reaction of Y-tagged HRPs and chemical polymerization of HRP in the other reports, the TL-mediated cross-linking reaction of the Y-tagged HRPs provides site-selective polymerization at the Y-tags and mild reaction conditions, resulting in retaining the catalytic activity of the resulting HRP polymers. The use of a Y-tag can substitute the use of mediators in laccase-catalyzed protein cross-linking reactions. The utilization of tyrosine coupling for new covalent bond formation resulted in active and highly functional HRP-based polymers, which could be further extended to the design of new biomaterials and bioconjugates applicable to biotechnology.

In Chapter 4, a novel strategy for controlling the structure of protein polymers by utilizing

tyrosine-containing loop peptide, Y-Loop, was explored. The Y-Looped BAP, BAP-Loop-Y, formed linear polymers, whereas BAPs fused with a *C*-terminal Y-tag showed irregular shapes in the SPM analysis. The sterically confined structure of the Y-Loop and a closely cross-linked BAP-Loop-Y caused steric hindrance around the cross-linked tyrosine residues at Y-Loops and prevented further cross-linking reactions and branch formations. Furthermore, linear BAP-Loop-Y/pG$_2$pA-Y copolymers that were prepared from the copolymerization reaction of BAP-Loop-Y and pG$_2$pA-Y showed excellent functionality and activity as protein probes in an OVA-detecting ELISA. These results suggested that the linear structure of the BAP-Loop-Y/pG$_2$pA-Y copolymers allowed them to pack better on the anti-OVA IgG-immobilized surface than the globular BAP-Y/pG$_2$pA-Y copolymers. Interestingly, the absorbance of the BAP-Loop-Y/pG$_2$pA-Y copolymers that were prepared with higher molar ratios of reporter enzyme to IgG-binding protein (BAP-Loop-Y to pG$_2$pA-Y) increased across the whole range tested. The shape of the protein polymers could be the most important factor in improving the functionality and activity of protein polymers. This newly proposed linear polymerization of BAP strategy can contribute to the further development of functional protein polymers for specific applications in biotechnology, biomedical sciences, bioimaging, and diagnostics.

The development of protein polymers will contribute in a range of applications that apply proteins or their processes. The protein polymerization reaction is one of interesting and promising approaches to improve the functionality and activity of protein in bioprocesses. The researchers can design the desired structure and characteristics of the protein polymers for their designated reactions. The protein polymerization reaction also will open further development of artificial proteins, for example, polymeric bifunctional proteins for specific applications. Protein polymers also applicable not only as protein probes in diagnostic applications but also in bioimaging and biosensors. Protein polymerization can be an interesting strategy for preparing an effective antigen for vaccine development. Although there are no sufficient reports regarding the response of immune system against polymeric antigens, this strategy is worth pursuing. The advances in the research and development of antibody-drug conjugates (ADCs) needs some enzymes to connect the antibody and drugs. Polymeric ADCs can become a novel approach in the development of new ADCs. Other enzymes that used in protein polymerization reaction are also good candidates for preparing ADCs. Therefore, the study of protein polymerization reaction by using a laccase reported in this dissertation will contribute to the further development of functional protein polymers in the future.