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学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称　博士（工学）　氏名　ファン　ティ　ヒエン

学 位 論 文 題 名

Expanding Substrate Scope of Sequence-regulating Polyhydroxyalkanoate Synthase for Block Copolymer Synthesis
（配列制御型ポリヒドロキシアルカン酸合成酵素の基質範囲を拡大しブロック共重合体を合成する）

In this study, I investigated the engineering of sequence-regulating polyhydroxyalkanoate (PHA) synthase to expand its substrate scope and synthesized novel block PHA copolymers by utilizing the engineered enzymes.

Chapter 1 summarizes the background on PHA biosynthesis, and their physical properties. PHAs are biobased polyesters attracting much research interest. The improvement of their physical properties is needed to expand the range of applications. PHA synthase plays a central role in the PHA biosynthesis. The function and classification of PHA synthases are introduced. Sequence-regulating polyhydroxyalkanoate (PHA) synthase PhaCAR is a unique enzyme that spontaneously synthesizes block copolymers from the mixture of substrates. Block copolymers composed of segments with distinct properties can exert useful and characteristic physical properties. For example, poly(2-hydroxybutyrate)-b-poly(3-hydroxybutyrate) P(2HB)-b-P(3HB) exhibited elasticity, whereas P(2HB-ran-3HB) is stretchable but not elastic. To expand the structure of PHA block copolymers, I designed a new block copolymer composed of flexible medium-chain-length (MCL) PHA and rigid polylactate (PLA) segments. A key to achieve this goal is the function of PHA synthase. The methods for evolutionary engineering of proteins to acquire the desired functions are introduced.

Chapter 2 describes the site-directed mutagenesis of PhaCAR to enhance the activity toward a MCL substrate 3-hydroxyhexanoyl-CoA (3HHx-CoA). PhaCAR has possessed broad substrate scopes that recognize artificial monomer 2-hydroxyacyl (2HA)-coenzyme (CoA), SCL monomer, but activity toward 3-hydroxyhexanoyl (3HHx)-CoA was weak. To improve the drawback, I performed site-directed saturation mutagenesis at position 314, which is previously identified hot spot adjacent to the catalytic center C315, and found that FH exhibited increased activity toward 3HHx-CoA compared with the parent enzyme. In addition, F314H (FH) synthesizes the P(3HHx) homopolymer in Escherichia coli cells expressing it. This finding enabled the synthesis of a new PHA block copolymer, P(3HHx)-b-P(2HB), in which the presence of a covalent linkage between the polymer segments has indicated via solvent fractionation.

In Chapter 3, PLA segment, which is immiscible with PHA, was introduced to synthesize a new type of PHA-PLA block copolymer by means of combinational effects of beneficials mutations. As a result, synergy of two beneficial mutations, N149D (ND) and FH, was found for D-lactate (LA) incorporation. The PhaCAR variants synthesized PDLA homopolymer segment in copolymer P(3HB)-b-PDLA and P(3HHx)-b-PDLA with molecular weight 105 in the order of magnitude. The biosynthesis of the high-molecular-weight PDLA segment was achieved for the first time.

Chapter 4 describes the attempt to isolate new beneficial mutant of PhaCAR by means of random mutagenesis to acquire the activity toward 3-hydroxyoctanoyl (3HO)-CoA. 3HO units enhanced flexibility of materials more effectively than 3HHx units. I explored the screening condition on fluorescent dye-containing agar plates for the directed evolution of PhaCAR to increase the activity toward 3HO-CoA. Initial attempts of random mutagenesis and screening assays identified several candidates of beneficial mutations. Although the mutants exhibited higher incorporation of 3HHx only, I constructed a basis of screening system for expanding substrate scope of PhaCAR toward creation of brand-new MCL-SCL block copolymers.

Chapter 5 summarizes the study and discuss the future perspectives.